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A STUDY OF THE MALE GENITALIA OF CANADIAN SPECIES OF PENTATOMIDAE[1]

BY ALEX. D. BAKER

IX. Specific Descriptions of the External Male Genitalia*

Brochymena quadripustulata

(Fabricius, Syst. Ent., p. 704, 1775, *Cimex.*) See Fig. 25, 26, 27, 28, 29.

General Features. Genital segment capable of retraction within the abdomen until the latero-caudal tips are in line with the posterior lateral edges of the seventh segment. Contained organs within the genital-cup clearly visible from the caudal aspect. Genital segment broadly and evenly convex dorsad. The ventral area has a gradual concave region in the midventral portion. Lateral tips obtuse and evenly rounded, projecting but little. Viewed vertically the genital segment widens gradually and evenly to the posterior. Width of genital segment about 1.8 mm.

External Surface of Genital Segment. Below the ventral border of the cup there is a gradually depressed area extending the whole width of the border and broadest medianly. This area is destitute of any vestiture. The region latero-ventrad of the above area is thickly covered with long upright hairs directed ventrad and caudad. These hairs continue dorsad around the lateral tips to the dorsal and lateral areas of the segment, but becoming very sparse on dorsal mesal area, the hairs being thickest along the lateral and posterior portions of the dorsal border of the cup. Pruinose, eighth segment not normally visible.

Borders of Genital-cup. The dorsal border, viewed dorsad, forms a right angle from which the apex has been removed, the latter part being comparable to the cephalic region. Viewed caudad the dorsal border is smoothly sinuate until it descends slightly to meet and form the even arch (superior ridge) over the proximal portion of the proctiger. Viewed ventrad, the ventral border is deeply concave, with a distinct but gradually rising projection from the median region. The tips show as evenly rounded areas. Lateral angles of the borders of the cup strongly acute, running subparallel to each other for a short distance. Ventral border becomes subcrenulate and both dorsal and

[1] *Concluded from February issue.*

* *Only those species are described of which there was an abundant supply.*

ventral margins laterad to the claspers are clothed with hairs shorter than those already described, but considerably denser. In the depressed median region of the ventral border the vestiture of hairs becomes sparse and very short.

The Claspers. Simple, broadened distad, polished, inner surfaces broadly flattened and smooth. Margins of ental surface rounded and forming distinct even border. Caudal aspect of ectal outline evenly convex, except for short concavity in latero-ventral region. Ectal outlines usually obscured by dense vestiture arising from the ventral border of the cup.

The Proctiger. Longitudinal axis about 30° from the vertical. Tip smooth; basal portion differentiated from the remainder. Dorsal two-fifths smooth and shining, denudate, with a median hyoidiform suture. Basal portion rougher and covered with fine pile. Division between the two regions sharp.

General. Noticeable differences appear to occur between individuals of this species. Two distinct types were noted (see Fig. 28 and 29). Type "A" from Covey Hill, Que., and type "B" from Macdonald College, Que. Genital-cup of "B" considerably less haired than "A". A caudal view of the ventral border of the genital-cup reveals differences near the median line. In "A" it arches upwards, in "B" it dips downwards. From the ventral aspect the ventral border tapers medianly to a distinct but obtuse point. In type "A" while this elevation is discernible, it is far less pronounced. In addition to these differences in the genitalia, it was later noticed, that, while the basal portion of the scutellum is swollen in both types, in "B" the elevation continues as a distinct ridge to the tip of the structure. In type "A" the elevated area does not extend backward over more than two-fifths of the visible portion of the scutellum. The genital variations are figured.

Brochymena affinis

(Van Duzee, Trans. Am. Ent. Soc. XXX p. 29, 1904.) See Fig. 30, 31 and 32.

General Features. Genital structures very sharply distinct from the preceding. Lower border of genital-cup more strongly produced caudad, so that the opening of the cup is more dorsad than caudad. Posterior lateral tips of genital segment greatly produced, so that it is not capable of being as completely retracted within the abdomen as the preceding. Genitalia normally concealed dorsad by wing tips. Maximum width of segment about 3.8 mm.

Borders of Genital-cup. Without any sharp projections; dorsal border, in general, arcuate but with caudo-ventral concavities cephalo-laterad to the claspers. Ventral border widely obtuse from caudal aspect. Dense regions of erect hairs on slight elevations of ventral border caudad to the claspers, but not as numerous as those of the preceding species.

External Surface of Genital Segment. Gradual depression of genital segment ventrad of the ventral border being similar to *B. quadripustulata* but wider to correspond to the greater width of the segment. Arrangement of hairs as in the preceding but coarser, denser and longer on latero-ventral region of segment. Pruinose, eighth segment normally concealed.

The Claspers. Simple, pointed and with a general twisted appearance. Ental surface margined, smooth and broad. Tapering to a point at distal end. Slight twisting of inner surface from mesad to meso-caudad. Lateral surface in outline shows two concavities with a gradual raised point between, which is directed laterad. Organs smooth, black and shining.

The Proctiger. Strongly arched longitudinally. Tip directed ventrad. Basal area smooth and shining for a short distance, merging gradually into an atomarius region which has sparse fine hairs. Proceeding distad, the structure

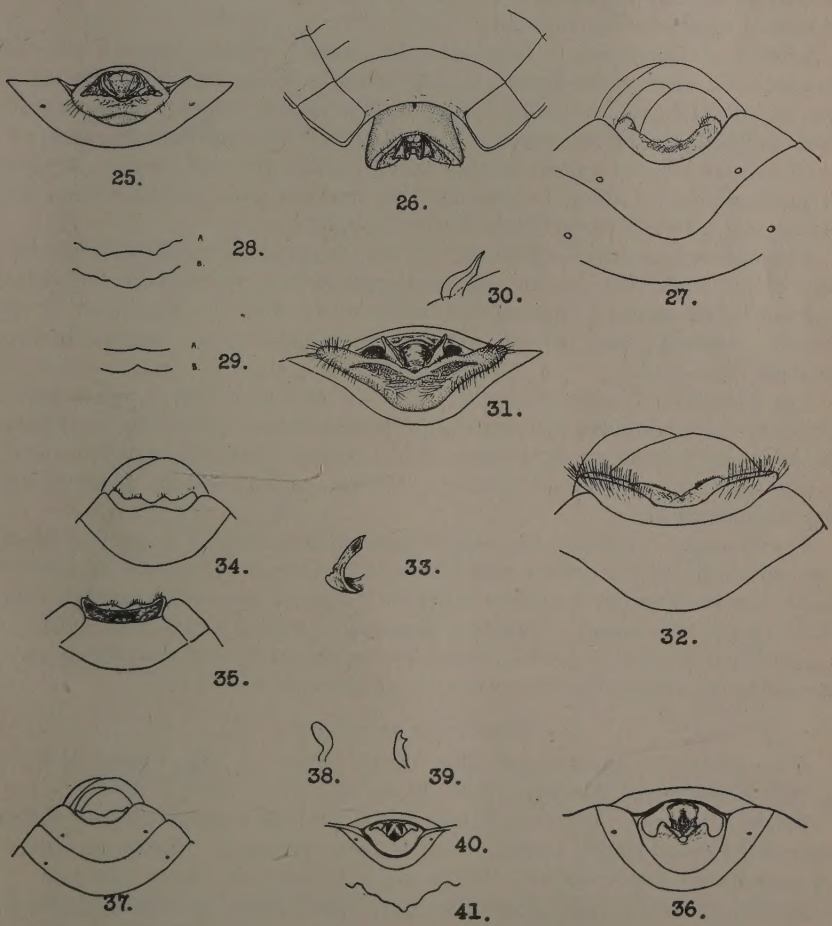


FIG. 25-29. *Brochymena quadripustulata Fabr.* FIG. 25. *Caudal aspect. (type B.)* FIG. 26. *Dorsal aspect.* FIG. 27. *Ventral aspect.* FIG. 28. *Caudal aspect of median portion of ventral margins.* FIG. 29. *Ventral aspect of median portion of ventral margins.*

Fig. 30-32. *Brochymena affinis Van D.* FIG. 30. *Caudal aspect of dextral clasper.* FIG. 31. *Caudal aspect.* FIG. 32. *Ventral aspect.*

FIG. 33-36. *Peribalus limbolarius Stal.* FIG. 33. *Caudal aspect of dextral clasper, with border of genital-cup removed.* FIG. 34. *Ventral aspect.* FIG. 35. *Dorsal aspect.* FIG. 36. *Caudal aspect.*

FIG. 37-41. *Trichopepla semivittata Say.* FIG. 37. *Ventral aspect.* FIG. 38. *Caudal aspect of dextral clasper.* FIG. 39. *Ental aspect of dextral clasper.* FIG. 40. *Caudal view.* FIG. 41. *Caudal aspect of ventral border of genital-cup.*

again becomes smooth and shining continuous to tip. Tip with a slight transverse depression.

Peribalus limbolarius

(Stal. Enum. Hemip., 11, p. 34, 1872.) See Fig. 33, 34, 35 and 36.

General Features. Opening of the genital-cup dorso-caudad. Genital segment capable of retraction until the caudal extremities are in line with the lateral caudal borders of the penultimate segment, *i.e.*, of the connexivum. As the seventh tergum does not extend as far caudad as the connexivum the interior of the cup is plainly visible when the wing tips are removed. Width of genital segment about 1.57 mm.

Borders of Genital-cup. Dorsal border largely hidden beneath seventh tergum and, from caudal aspect, broadly arcuate except for very slight elevation in lateral region. Ventral border vertical and with two prominent truncated projections directed dorsad. Lateral corners of projections rounded, but ental corners pointed and projecting mesad leave a rounded opening ventrad in median area. Lateral borders broadly rounded from caudal aspect, but acutely angled when viewed from the dorsal aspect.

External Surface of Genital Segment. With the exception of the external surface of the projections on the ventral border already referred to, the visible portion of the segment is punctated, in harmony with the remainder of the insect's abdomen, and covered with sparse minute hairs. Surface of projections immaculate.

The Claspers. Simple, bladelike with tip ending in sharp acute point. Strongly curved on basal half, the tips projecting dorsad and somewhat laterad. Mesal margin crenulate near base. Ectal margin shallowly concave distad, giving rise to an obtuse angle laterad. Margins smooth, except as above, and slightly margined.

The Proctiger. Vertical, except at tip, which rests parallel to the subvertical ventral border of the genital-cup. Narrowly margined at base, remainder of surface smooth except a median inverted V-shaped area which extends four-fifths of the way basad. This is a diagonally striated and faintly margined longitudinal invagination which, being less highly sclerotized than the remainder of the exposed surface, permits a dorsal flexion of the organ.

Trichopepla semivittata

(Say, Heter. N. Harm., p. 9, 1832; Fitch reprint, p. 766; Compl. Writ. 1, p. 322, *Pentatoma.*) See Fig. 37, 38, 39, 40 and 41.

General Features. Posterior lateral tips of seventh segment projecting considerably beyond genital segment when latter is retracted. Maximum opening of genital-cup dorso-caudad. Width of genital segment about 1.14 mm.

Borders of Genital-cup. Dorsal border, viewed dorsad, normally hidden by seventh tergum. Arched from caudal aspect descending slightly to form a secondary arch over the base of the proctiger, which bears a slight median depression. Ventral lateral angles acute and margins subparallel for a short distance mesad. (Variance and slight asymmetry in latter feature.) Ventral border of cup subvertical. Ventral border, in general, from caudal aspect,

V-shaped with a rounded median notch. Rounded, but abrupt ventral curvation, in dorso-lateral region and with intermediate regions sinuate. From ventral aspect the segment appears pointed at caudo-lateral tips and with two gradual but prominent swellings laterad to a slight median notching.

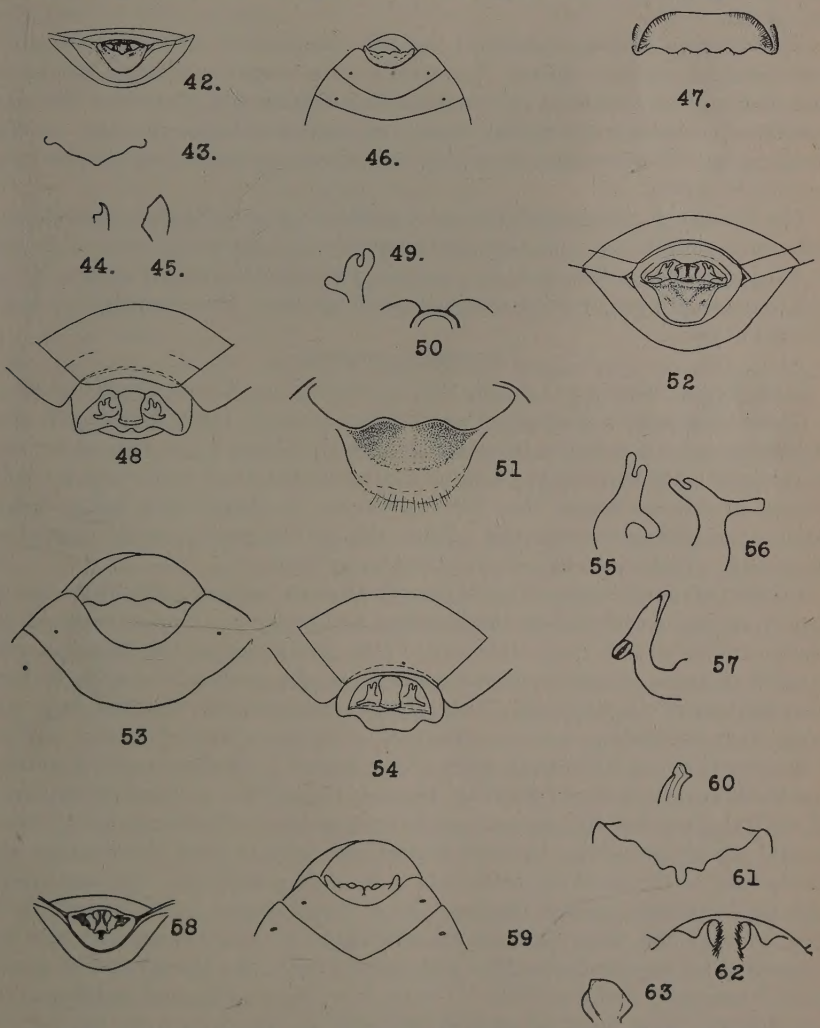


FIG. 42-47. *Trichopepla atricornis Stal.* FIG. 42. *Caudal aspect.* FIG. 43. *Caudal view of ventral border.* FIG. 44. *Caudal view of tip of sinistral clasper.* FIG. 45. *Ental view of sinistral clasper.* FIG. 46. *Ventral view.* FIG. 47. *Dorsal view of borders of genital-cup.*

FIG. 48-51. *Chlorochroa uhleri Stal.* Fig. 48. *Dorsal view.* FIG. 49. *Caudal view of sinistral clasper.* FIG. 50. *Caudal view of dorsal margin of genital-cup with procliger removed.* FIG. 51. *Caudal aspect of ventral border of genital-cup showing ventral "lip".*

FIG. 52-57. *Chlorochroa sayi Stal.* FIG. 52. *Caudal view.* FIG. 53. *Ventral aspect.* FIG. 54. *Dorsal aspect (with wing tips removed).* FIG. 55. *Caudal view of dextral clasper.* FIG. 56. *Ental view of dextral clasper.* FIG. 57. *Dextral view of dextral clasper.*

FIG. 58-63. *Carpocoris remotis Horv.* FIG. 58. *Caudal aspect.* FIG. 59. *Ventral aspect.* FIG. 60. *Caudal view of dextral clasper.* FIG. 61. *Caudal view of ventral border of genital-cup.* FIG. 62. *Dorso-caudal view of structures adjacent to base of procliger.* FIG. 63. *Ental aspect of sinistral clasper.*

External Surface of Genital Segment. Slight irregular depressions below ventral margin of the genital-cup ventrad to projections. Exposed surface of segment punctate, conforming to remainder of abdomen including the long fine pubescence which is found on all parts of the body of insects belonging to this genus.

The Claspers. Simple, polished, broadly flattened from inner aspect and with smoothly curved outline. On ectal surface sharp acute tooth about one-half distance towards base. Distad and basad from this projection the ectal outline is concave from caudal aspect, except for a slight swelling towards basal region. From caudal aspect, tips are acutely pointed; from ental aspect, broadly rounded.

The Proctiger. General features very similar to that of *Peribalus limbolarius.* Subvertical in position with tip bent dorsad to meet the vertical ventral border of the genital-cup. Less heavily sclerotized inverted V-shaped area on distal half of organ present permitting upward articulation. Base slightly margined. Devoid of hairs.

Trichopepla atricornis

(Stal, Enum. Hemip., 11, p. 34, 1872.) See Fig. 42, 43, 44, 45, 46 and 47.

General Features. Opening of genital-cup as in *T. semivittata* except that slightly less of cup is visible from caudal aspect. Shows many general features in common with preceding. Genital segment capable of retraction into abdomen to a lesser degree than former species, the lateral tips being closely adjacent to tips of connexivum. Wing tips do not project as far caudad as preceding. Width of segment but little over 1 mm.

Borders of Genital-cup. Dorsal border smooth and straight, from dorsal aspect, in region cephalad to the proctiger and claspers. Cephalo-laterad the border curves evenly caudo-laterad. From caudal aspect the dorsal margin consists of three subequal smooth concavities, the median concavity arching over the base of the proctiger. Lateral inner angles acutely rounded, but more open than preceding species. Ventral margin as figured, being roughly V-shaped from caudal aspect, with apex rounded. Median angle of ventral border more obtuse than preceding, being at least 120°. Lateral terminations of ventral margin ending in two rounded concavities. Outline of margin from caudal aspect bisinuate; between lateral and median area the number and abruptness of the swellings being less than in *T. semivittata.* Ventral border also thicker except median portion. From dorsal aspect, ventral border with tips rounded and three right-angled notches in the median region which are connected by regular convexities and, laterad, with the lateral tips by a successive convexity and concavity. Concavities, convexities and notches all of equal depth, there being no general concavity or convexity from the vertical aspects.

External Surface of Genital Segment. In general, surface pilose and punctated in conformity with remainder of abdomen. Shallow depression from concavities of ventral border within the caudo-lateral tips of genital segment. This depression broadens and decreases in depth meso-ventrad. Depression results in slight ridging meso-ventrad from tips of genital segment.

The Claspers. Simple, pointing subdorsad, smooth, flattened on inner surface, obtuse angle at tip, obtusely biangulate caudad and evenly curved cephalad. From caudal aspect thick and stout, except at tip which ends in a dorso-lateral curved point with a deep cephalo-caudal groove just basad from the tip on the ectal surface.

The Proctiger. Longitudinally curved caudo-ventrad with tip turning caudad to meet the inner ventral border of the genital-cup. Umbonate, smoothly and less highly sclerotized distad to the median knoblike process. Umbone and adjacent lateral area punctate and sparsely haired. Basal area punctate and irregularly transversely wrinkled. Border of tip smooth.

Chlorochroa uhleri

(Stal, Enum. Hemip., 11, p. 33, 1872.) See Fig. 48, 49, 50, 51 and 18.

General Features. Opening of genital-cup dorso-caudad. Details within cup less visible from caudal than from dorsal aspect (*i.e.*, with wings removed). A stout broadly convex genital segment with no sharp angles. Width of segment about 1.86 mm.

Borders of Genital-cup. Dorsal border dorsally arched over base of proctiger with two deep dorso-cephalic concavities laterad. Superior ridge absent. Lateral angles at least slightly acute from all aspects, but not sharp. From both dorsal and caudal aspects the ventral margin is concavely bowed from tip to tip, except for an elevated mesal area with rounded sides and a central or median rounded depression.

External Surface of Genital Segment. Area ventrad of ventral border considerably depressed, and below, extended caudo-ventrad to form a secondary border. Secondary border convex in outline from dorsal and caudal aspects, and set with a numerous but not dense array of hairs. Ectal angles of tips are haired in a like manner to above.

The Claspers. Three branched, with basal stem broad and exposed. Stem directed subdorsad, later curving gradually dorso-cephalad. Midway from point of articulation, a thumblike process is given off from ectal region which projects laterad and slightly caudad. Main stem continues broadly dorso-cephalad, dividing at its distal extremity to form two connivent lobes or branches. Ecto-distal branch bluntly pointed, while tips of remaining projections are broadly rounded. Regions distad to lateral thumblike projection with fine long erect hairs.

The Proctiger. General position subhorizontal. Tip smooth and rounded but gradually constricted in middle. Grooved medianly except at tip. Basal portion and region near tip gradually swollen. Destitute of hairs except for a fine fringe around anal area.

Chlorochroa sayi

(Stal, Enum. Hemip., 11, p. 33, 1872.) See Fig. 52, 53, 54, 55, 56 and 57.

General Features. Size and general shape of genital segment very similar to *C. uhleri.* Cup more widely open dorsad than caudad. Lateral tips smoothly rounded, protruding but little. Secondary lip extending further caudad than lateral tips. Lateral tips extending but little beyond posterior tips of connexivum.

Borders of Genital-cup. Ventral border, from dorsal aspect, nearly straight. Dorsal and lateral margins continuous and evenly bowed. Ventral border concealing more of genital structures than preceding. Lateral angles of cup acute from all aspects, sharply from dorsal aspect, but roundly from caudal aspect. Dorso-lateral border infolded ventrad, forming an uneven inner outline. Ventral margin swollen caudad to the latero-distal margins of the proctiger, becoming gradually thinner mesad and laterad. From caudal aspect, these swellings give the ventral margin a sinuous outline.

External Surface of Genital Segment. Punctate, hairy and similar generally to *C. uhleri.* Region ventrad of ventral border depressed, forming a prominent secondary margin or lip below. This secondary margin extends further caudad and also further ventrad than the homologous structure in *C. uhleri.* The depressed area has but fine pile at margins, near adjacent ventral border, and is usually impressed with irregular transversely curved wrinkles. In median region, just ventrad to ventral border, there is a small, but prominent black spot.

The Claspers. Three branched. Main stem twisted dorso-laterad, giving off first branch which is prominent, truncate and extends caudo-laterad *in situ.* This branch is the most prominent of the three and is slightly and gradually constricted between base and tip. Remaining branches extend dorso-cephalad, to end in rounded tips; the ental projection longer than that of the ectal. Region basad to the two terminal branches, stout and at least as broad as the distance between the two apices. All the visible portions of the claspers are clothed with fine erect hair.

The Proctiger. General position subhorizontal. Margin of tip even and slightly thickened. Shallowly restricted laterad, and with a slight median depression between distal and basal margins. Basal area widest part of structure, but constricted at immediate point of union with segment. Sparsely and finely haired. Basal half usually darker in color than distal region.

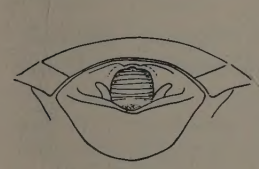
Carpocoria remotis

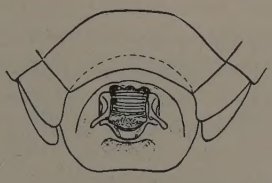
(Horvath, Ann. Mus. Matl. Hung., v, p. 296, 1907.) See Fig. 58, 59, 60, 61, 62 and 63.

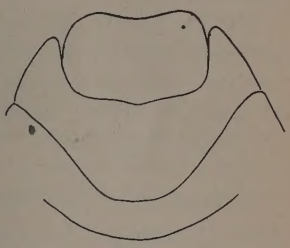
General Features. Genital-cup open to an equal degree to caudal and dorsal aspects, but wing tips normally hide the genital structures completely. Genital segment capable of retraction until its lateral tips are cephalad to caudal tips of the connexivum. Tips acutely rounded. Width of genital segment about 1.57 mm.

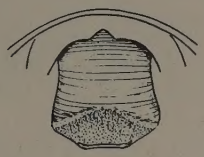
Borders of Genital-cup. Dorsal border mildly arcuate from dorsal and caudal aspects, except for two right-angled projections laterad to the base of the proctiger. These structures project into the genital-cup approximately caudo-mesad. Lateral angles of cup rounded, but acute. From caudal aspect, ventral border obtusely V-shaped (108°). Deeply notched on median line, pronounced concavities immediately laterad, and, proceeding laterad, two shallow depressions, mesad of which the border is thickened. All of ventral border set with erect hair, which becomes sparse towards the lateral tips.

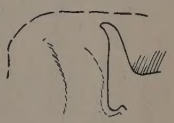
External Surface of Genital Segment. Exposed surface irregularly and transversely wrinkled. Region ventrad of ventral margin depressed and with short sparse hairs. Region immediately ventrad of median notch of ventral border only thinly sclerotized and semitransparent.

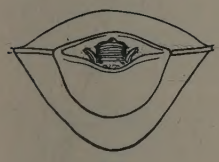


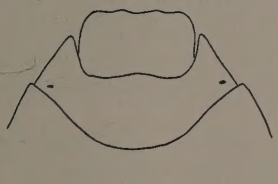


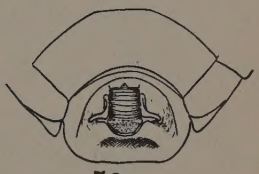


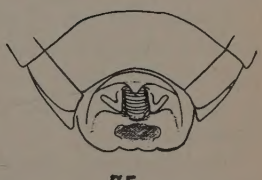


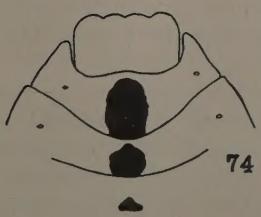












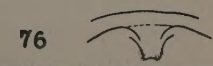


FIG. 64-68. *Euschistus euschistoides Vall.* FIG. 64. *Caudal view.* FIG. 65. *Dorsal view (with wing tips removed).* FIG. 66. *Ventral view.* FIG. 67. *Ental aspect of sinistral clasper.* FIG. 68. *Dorso-caudal view of superior ridge and procliger.*

FIG. 69-72. *Euschistus conspersus Uhl.* FIG. 69. *Caudal aspect.* FIG. 70. *Ventral aspect.* FIG. 71. *Ental view of sinistral clasper (with procliger removed).* FIG. 72. *Dorsal aspect (with wing tips removed).*

FIG. 73-76. *Euschistus tristigmus Say.* FIG. 73. *Caudal aspect.* FIG. 74. *Ventral aspect.* FIG. 75. *Dorsal aspect (with wing tips removed).* FIG. 76. *Details of superior ridge, etc.*

The Claspers. Simple, without branches or any prominent projections. Broad and flattened on inner surface. Sharply margined or keeled on caudo-mesal border, cephalic border, caudo-lateral border, and to a lesser degree, at apex. This renders the caudal side deeply sulcate. Movement of organs dorso-mesad.

The Proctiger. General position more vertical than horizontal. With two margined, lateral depressed areas which give the organ the appearance of being gradually broader towards its tip. Basal region smooth and shining, remainder of structure densely haired and uneven. Not a highly sclerotized structure. Tip rounded and entire.

Euschistus euschistoides

(Vollenhoven, Versl. Med. Kon. Akad. Wetens. Amst., Ser. 2, ii, p. 180, 1863, *Diceraeus.*) See Fig. 64, 65, 66, 67, 68 and also Fig. 8, 9, 156, 158, 160 and 161.

General Features. Genital-cup widely open dorsad, but normally hidden by wing tips. Segment broadly rounded and without any abrupt projections. When retracted the ventral border conforms fairly closely to the general abdominal outline. Definite lateral tips absent.

Borders of Genital-cup. Dorsal border, in general, evenly arched from dorsal and caudal aspects. Superior ridge present as a prominent caudal extension ventrad to the dorsal border proper, forming a protective yoke over the base of the proctiger. Superior ridge evenly arched, except for a sharp right-angled notch mediad, as figured. Definite inner lateral angles wanting, the lateral margins merging gradually into the caudal liplike structure of ventral border. Ventral border, from all aspects, with smooth margins and broadly rounded obtuse lateral angles, and in general, flat, broad and considerably extended to the posterior.

External Surface of Genital Segment. Undifferentiated from remainder of venter. Ventral border of cup widely explanate, as described above, which gives the region below the margin a concave outline when viewed from the lateral aspect. Denudate.

Surface Within Genital-cup. Inferior ridge present as two transverse broadly rounded lateral carinae arising strongly just caudad to the claspers and running caudo-laterad to merge with the ventral lip. Mesad the ridge is arched caudad and it is against this portion that the tip of the proctiger rests *in situ.*

The Claspers. Simple, without branches or any prominent projections. From caudal aspect, curved evenly and smoothly dorso-laterad to tapering rounded tips. Basal area considerably broadened and extends cephalad visibly to base of proctiger. From ental aspect tips project dorso-caudad and are broadly rounded distad, with an erect fringe of long hairs on basal cephalic border.

The Proctiger. Declinate; basal region smooth, evenly and finely banded. Differentiated near tip, which is incrassated and roughened, being without any evidence of transverse banding. Distal area clothed with fine scattered hairs

and sharply marked off from the major smoother basal region which precedes it. The basal attachment of the organ is not visible externally.

Euschistus conspersus

(Uhler, Trans. Md. Acad. Sci., i, p. 388, 1897.) See Fig. 69, 70, 71 and 72.

General Features. Genital-cup widely open dorsad, but normally hidden by wing tips. Cup not capable of retraction beneath seventh tergum. Width of genital segment about 1.9 mm. From edge of "lip" to base of proctiger about 1.14 mm.

Borders of Genital-cup. Dorsal border, from caudal and dorsal aspects, broadly and evenly rounded. Superior ridge present and sharply and right-angularly notched medianly. Ventral border forms a distinct liplike structure. Definite lateral angles wanting, the lateral margins gradually merging into the ventral border.

External Surface of Genital Segment. Denudate, slightly and evenly depressed below ventral border of lip. Concolorous on ventral side, matching the remainder of venter. Exposed dorsal areas darker, to match the darker upper surface of abdomen, except medium dorsal portion of lip, which is the same color as the venter.

Surface Within Genital-cup. Inferior ridge present as a prominent elevated transverse structure within ventral border, forming a fence or border around the tip of the proctiger. Ridge broadens laterad to gradually merge with the lateral and caudo-lateral interior surfaces of the genital-cup. The presence of this ridge results in a deep depression cephalad to the ventral margin.

The Claspers. Simple and sharply deflexed dorsad, when viewed from ental aspect, and very easy to distinguish from those of other species of the same genus. Extreme tip bluntly pointed and curved slightly caudad. Erect, long, dorsally directed hairs on dorsal border of basal region, extending but little beyond the region of dorsal curvature. From ental aspect, caudal margin broadly but distinctly knobbed at base of dorsally directed portion of structure. From caudal aspect, organs evenly curved from a dorsal to a dorso-lateral direction.

The Proctiger. Very similar to *E. euschistoides*, etc., but smaller in proportion to size of genital segment and with incrassated area extending but little farther basad in median area than in lateral regions.

Euschistus tristigmus

(Say, Heter. N. Harm., p. 4, 1831; Fitch reprint, p. 758; Compl. Writ., i, p. 314, *Pentatoma*.)

See Fig. 73, 74, 75, 76, 77 and also Fig. 11, 12, 13, 14, 19, 20, 21 and 157.

General Features. Genital-cup widely open dorsad, but normally hidden by wing tips. Cup not capable of retraction beneath seventh tergum. Width of genital segment about 1.7 mm. Cup with caudal lip. Lateral tips rounded.

Borders of Genital-cup. Dorsal border evenly rounded. Superior ridge present and prominent. This ridge is unnotched and more prominent than the homologous structures described for other species belonging to this genus. These specific differences are figured (and from which the variations between

species can be easily noted). Ventral border with three equal and sharp indentations, as viewed from vertical aspects. From caudal aspect, lip considerably thicker laterad than in median area, where it is restricted and deeply concave. Lateral angles absent.

External Surface of Genital Segment. Color markings very similar to those of *E. conspersus*. From lateral aspect, ventral surface strongly concave.

Surface Within Genital-cup. Inferior ridge present and widening laterad to merge gradually with the lateral and caudal borders of the cup. Caudad to the inferior ridge is a deep and broad depression bounded caudad by the ventral margin of cup. The borders of this depression are lined with erect hairs, which project towards its centre.

The Claspers. Simple, from ental aspect, sharply bent dorso-cephalad. Tips smoothly and broadly rounded. Viewed from caudal aspect, there is but little lateral curvature and tips project directly dorso-laterad. Hairs on cephalic basal border similar to those of *E. conspersus*. Knob at base of bent portion. The direction of the tips as viewed from the lateral or ental aspects renders this species easily distinguishable from the other species of this genus herein studied.

The Proctiger. Very similar to that of *E. conspersus*, but not extending as far caudad nor as strongly curved.

Euschistus variolarius

(Palisat-Beauvois, Ins. Rec. Afr. Am., p. 149, pl. Hemip. 10, Fig. 6, 1805, *Pentatoma.*) See Fig. 78, 79, 80.

General Features. Genital-cup widely open dorsad, but normally hidden by wing tips. Opening of cup not generally retracted beneath seventh tergum. Width of genital segment about 2.14 mm. Cup with distinct lip, as in other species of this genus.

Borders of Genital-cup. Dorsal border very similar to that of *E. euschistoides;* also the notched superior ridge, which is present over the base of the proctiger. Ventral border with slight indentations, not as strongly marked as those of *E. tristigmus*, but slightly more so than those of *E. conspersus*. From caudal aspect, ventral border very similar in outline to that of *E. euschistoides*. Lateral angles absent.

External Surface of Genital Segment. Region below lip depressed, but not as strongly as in *E. tristigmus*. Ventral surface of segment of uniform color except for the presence of a large medio-basal rounded black spot which has been used as one of the chief distinguishing characters of this species. Dorsal surface, in general, dark, but lighter over most of lip and latero-caudal regions.

Surface Within Genital-cup. Inferior ridge present and widening laterad. Deep depression caudad to inferior ridge, but only shallow depression caudad and laterad. Erect hairs arising from inferior ridge and shorter hairs within caudal depression.

The Claspers. Simple. Bent dorso-caudad and similar in general outline to those of *E. euschistoides*, but rows of hairs on cephalic border usually extending farther distad.

The Proctiger. Shining, glabrous and banded, except distal portion which is similar to that region found in *E. euschistoides.* Stout, strongly arched transversely and longitudinally.

Euschistus ictericus

(Linneaus, Cent. Ins., p. 16, 1763, *Cimex.*) See Fig. 81, 82, 83, 84.

General Features. Genital-cup widely open dorsad, but normally hidden by

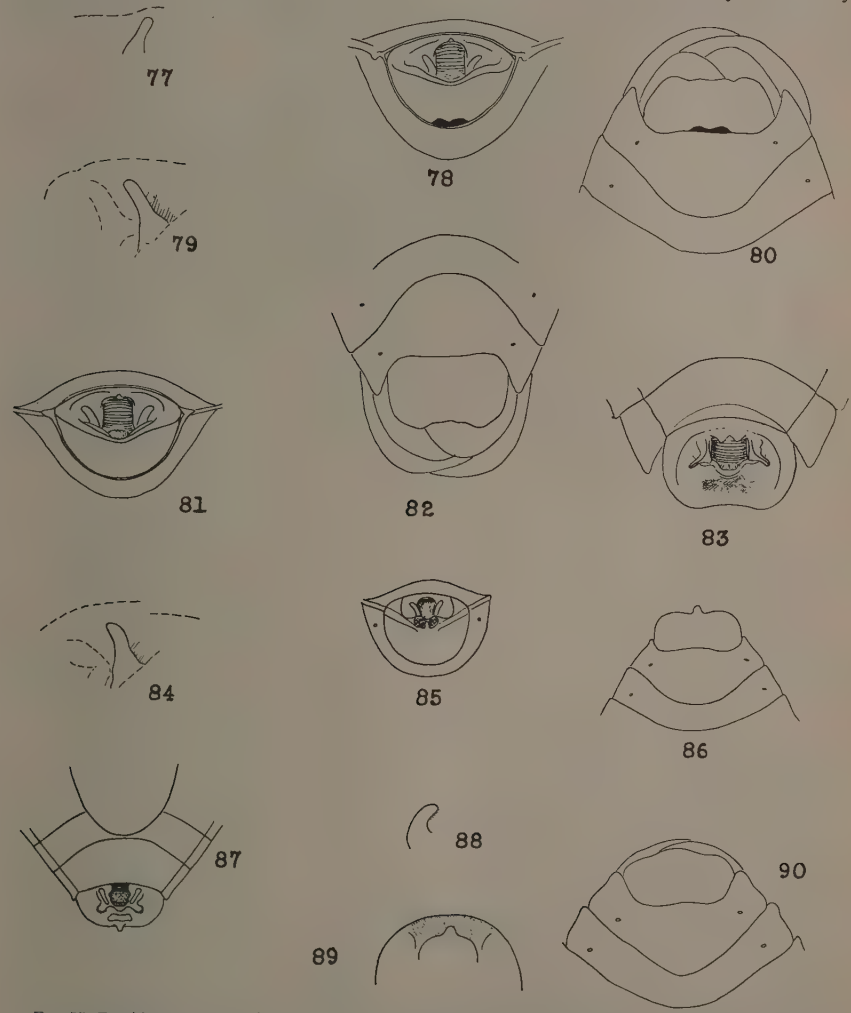


FIG. 77. *Euschistus tristigmus Say. Ental view of sinistral clasper.*
FIG. 78-80. *Euschistus variolarius P.B.* FIG. 78. *Caudal aspect.* FIG. 79. *Ental view of sinistral clasper in situ.* FIG. 80. *Ventral aspect.*
FIG. 81-84. *Euschistus ictericus Linn.* FIG. 81. *Caudal view.* FIG. 82. *Ventral aspect.* FIG. 83. *Dorsal aspect.* FIG. 84. *Ental view of sinistral clasper in situ.*
FIG. 85-89. *Coenus delius Say.* FIG. 85. *Caudal aspect.* FIG. 86. *Ventral aspect.* FIG. 87. *Dorsal aspect (with wing tips removed).* FIG. 88. *Ental view of tip of sinistral clasper.* FIG. 89. *Dorso-caudal view of the details of the superior ridge and dorsal border of genital-cup.*
FIG. 90. *Hymenarcys nervosa Say. Ventral aspect.*

wing tips. Width of genital segment about 2.14 mm. From base of proctiger to margin of lip about 1.28 mm. Tip of proctiger rests about 0.7 mm. from caudal margin of lip. Genital-cup with lip as found in other species of this genus. Tips broadly and evenly rounded. Lip usually projecting considerably caudad to connexivum tips.

Borders of Genital-cup. Dorsal border, in general, similar to that of *E. euschistoides*, but with superior ridge not as distinctly separated from border proper. Superior ridge strongly and right-angularly notched. Lateral angles wanting. Ventral border of cup with a single broad concavity.

External Surface of Genital Segment. From lateral aspect, concave in outline in region below lip. Ventral surface concolorous and in harmony with remainder of venter. Dorsal surface darker cephalad of dorsal border, but lateral and posterior regions similar in color to venter.

Surface Within Genital-cup. Inferior ridge present, broadening laterad and turning sharply caudad. (This latter feature appears to be quite specific.) The lateral extremities of inferior ridge visible within the cup from the caudal aspect, as figured.

The Claspers. Simple, bent dorso-caudad from horizontal basal portion. Similar in outline, from ental aspect, as that of *E. euschistoides* but more sharply rounded and bent very slightly caudad. Hairs on cephalic border present, sparse and not extending more than half way to tip from point of bend. From caudad aspect, slightly and smoothly curved laterad. General direction dorso-laterad.

The Proctiger. Similar to *E. variolarius*, but generally lighter in color.

Coenus delius

(Say, Heter. N. Harm., p. 8, 1831; Fitch reprint, p. 765; Compl. Writ., i.p. 320, *Pentatoma*.) See Fig. 85, 86, 87, 88, 89; see also Fig. 10 and 159.

General Features. An insect with well-marked specific genital characters. Genital-cup widely open dorsad, but with interior structures deeply imbedded. Cup not capable of being retracted beneath the seventh tergum except slightly, so as to conceal dorsal border. Width of genital segment about 1.19 mm. Genital-cup without definite lip. Wing tips do not extend caudad of genital segment. Lateral tips of segment broadly rounded and subcontinuous with connexivum when retracted. Convex dorsad and ventrad.

Borders of Genital-cup. Dorsal border strongly and evenly arched. Superior ridge present, prominent, and deeply but roundly notched medianly. Lateral and ventral borders elevated and broad, so that claspers and proctiger appear deeply imbedded within cup. Lateral margin continuous with inner margin of inferior ridge. Caudad of inferior ridge there is a deep medianly-ridged depression. From the caudal border of latter concavity, and continuous with the median ridge, rises a strong blunt spine which projects dorso-caudad. This median spine is visible from dorsal, caudal, and ventral aspects, and a specific character (Canadian forms). Inner margins of cup with erect hairs projecting inwards.

The Claspers. Simple, strongly and evenly curved cephalad. Tips rounded.

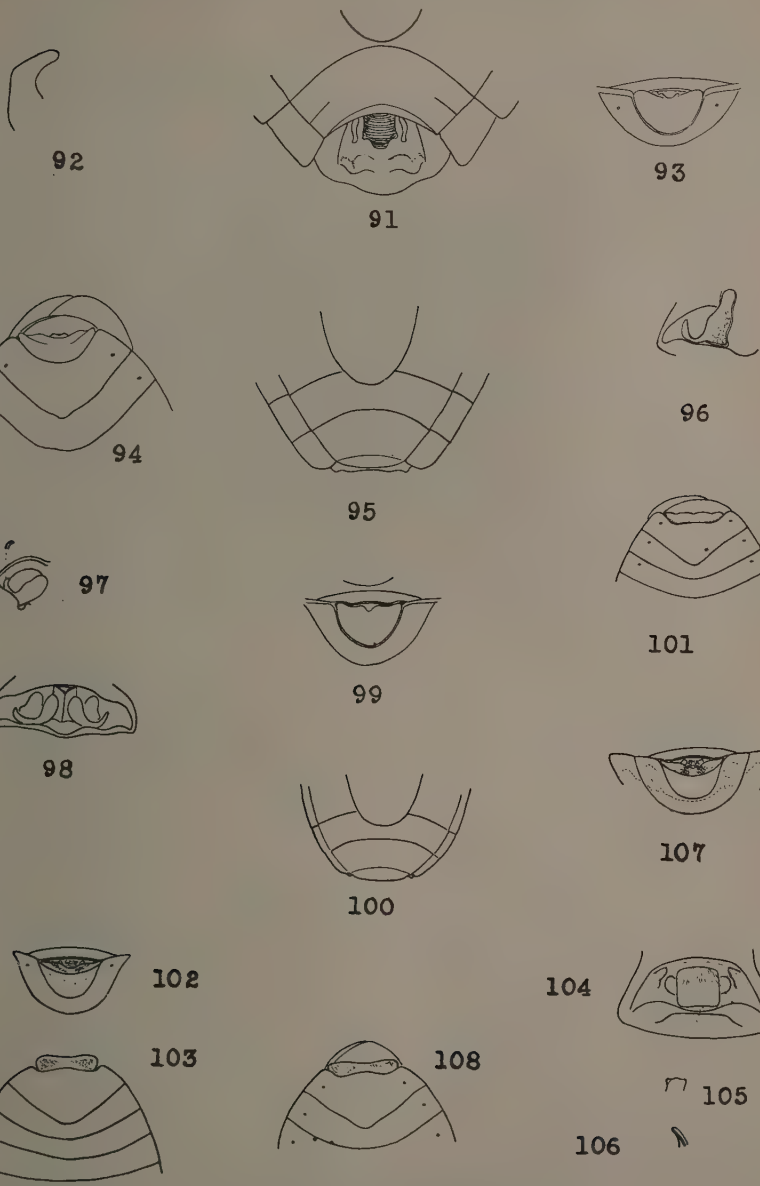


FIG. 91-92. *Hymenarcys nervosa Say.* FIG. 91. *Dorsal aspect (with wing tips removed).* FIG. 92. *Ental view of tip of sinistral clasper.*

FIG. 93-96. *Aelia americana Dall.* FIG. 93. *Caudal aspect.* FIG. 94. *Ventral aspect.* FIG. 95. *Dorsal aspect (with wing tips removed).* FIG. 96. *Subcaudal aspect of sinistral clasper within genital-cup.*

FIG. 97-101. *Neottiglossa undata Say.* FIG. 97. *Latero-dorso-caudal view of sinistral half of genital-cup showing details and relations of clasper and genital plate.* FIG. 98. *Dorso-caudal view of genital-cup (removed from beneath 7th tergum).* FIG. 99. *Caudal view.* FIG. 100. *Dorsal view (wings removed).* FIG. 101. *Ventral view.*

FIG. 102-106. *Cosmopepla bimaculata Thom.* FIG. 102. *Caudal view.* FIG. 103. *Ventral view.* FIG. 104. *Dorsal view of genital-cup.* FIG. 105. *Ental aspect of sinistral clasper.* FIG. 106. *Caudal aspect of sinistral clasper.*

FIG. 107-108. *Cosmopepla conspicillaris Dall.* FIG. 107. *Caudal view.* FIG. 108. *Ventral view.*

Distal cephalic margin with few erect short hairs. From caudal aspect, tips erect and but slightly curved ectad. From dorsal aspect, straight and directed approximately cephalo-laterad, with slightly swollen extremities. Surface smooth and polished.

The Proctiger. Deep, well-separated transverse grooves over basal half. Transverse grooves fewer but deeper than those found in *Euschistus* spp. Distal half roughened and thicker, with fine scattered hairs, which are absent on basal half. Tip uncertainly margined. Base of structure normally slightly concealed beneath seventh tergum and with tip extending but halfway across the visible dorsal portion of the genital segment.

Hymenarcys nervosa

(Say, Heter, N. Harm., p. 9, 1832; Fitch reprint, p. 766: Compl. Writ., i.p. 321, *Pentatoma.*) See Fig. 90, 91, 92.

General Features. Genital-cup widely open dorsad, but with anterior region normally somewhat concealed beneath seventh tergum, which results in the partial obscuring of the base of the proctiger and the distal extremities of the claspers. Width of genital segment about 2 mm. When fully retracted, about 1.3 mm. of segment normally visible caudad to seventh tergum. Wing tips normally cover genital-cup, but project but little caudad to it. Lateral tips rounded and not closely continuous with connexivum, but as much so as junctions between segments of abdomen proper. Genital-cup with a distinct lip.

Borders of Genital-cup. Dorsal border normally hidden beneath seventh tergum, but evenly arched anteriorly and dorsad. Superior ridge present and of even outline. Inferior ridge present, deeply and roundly notched medianly and laterally continuous with lateral angles of cup. Lateral and ventral borders of cup broadly thickened. Inferior ridge clothed with fine erect short hairs. From dorsal aspect, ventral margin broadly convex medianly and with slight broad concavities laterad.

The Claspers. Simple, strongly curved, so that tips point cephalad. Inner surface at point of bending even; outer surface more abruptly angled, but obtuse. Structures broader from ental than other aspects. From dorsal aspect they present a slightly sinuate appearance, but, in general, lie parallel to the median line. Hairs absent from those portions that normally project above plane of tip of proctiger. Structures smooth and glistening.

The Proctiger. Stout and strongly arched. Banded, smooth and polished over basal three-fourths. Spacing and depth of transverse grooving similar to that found on basal region of proctiger of *Coenus delius.* Distal region broader, except immediate tip, which is considerably narrowed, finely haired and punctate.

Aelia americana

(Dallas, List of Hemip., i, p. 223, 1851.) See Fig. 93, 94, 95, 96.

General Features. Genital-cup opening broadly but not deeply to dorso-posterior. Cup normally completely concealed beneath seventh tergum, so that only the ventral border is visible. In consequence, genital structures are

not usually visible externally. Width of genital segment about 1.3 mm. Maximum depth of opening of genital-cup about 0.3 mm., except for region immediately cephalad of claspers and proctiger, which measures about 0.43 mm. Lateral tips closely approximating tips of connexivum, but not continuous. Lateral tips acutely, but roundly angled. Lip absent.

Borders of Genital-cup. Dorsal border interrupted medianly by bases of claspers and proctiger. (In this feature one can see evidences of the formation of the genital segment by the upward and inward swelling of the sternum. The ninth tergum, if present, is probably represented by the membranous bands cephalad to the base of the proctiger.) Remainder of lateral portions of dorsal border strongly and evenly arched and covering the cephalic portion of the genital-cup. Lateral angles of cup roundly acute. Ventral border thickened, except near tips and median portion, where it is abruptly thinner. From caudal aspect, ventral border presents a rounded median notch.

External Surface of Genital Segment. Exposed portion of genital segment strongly flattened posteriorly, shallowly depressed in lateral regions, and, immediately below median notch of ventral border, depressed and denudate. Dorsal and lateral regions convex and destitute of vestiture, except for few fine short hairs near lateral angles of cup.

The Claspers. Spurred, main basal portion lying closely parallel to proctiger. Inner margin of basal region strongly elevated, giving it a distinct border which is continuous around caudal part. From caudo-lateral angle of basal portion there arises a strong spur, which curves laterad and then cephalad. Tip of spur darker in color than remainder of structure.

The Proctiger. Cariose, and set with irregular long pendant hairs. Attached dorso-cephalad to what appears to be the genital-cup proper, so that the organ has the appearance of arising exterior to the cup and hanging down within it.

Neottiglossa undata

(Say, Heter. N. Harm., p. 8, 1831; Fitch reprint, p. 764; Compl. Writ., i, p. 319, *Pentatoma.*) See Fig. 97, 98, 99, 100, 101.

General Features. Apparently closely related to *Aelia americana.* Genital-cup opens broadly, but not deeply, to dorso-posterior. Cup normally completely concealed beneath seventh tergum, so that only ventral border is visible from caudal aspect, and usually only the lateral tips of the genital segment from the dorsal aspect. Width of genital segment about 1.14 mm. Maximum depth of opening of genital-cup about 0.3 mm. Ventral lip absent. Lateral tips acutely rounded.

Borders of Genital-cup. Dorsal border without the median anterior notching over base of proctiger as in *Aelia americana.* Broadly and evenly arched, except for single gradual convexities cephalad to tips of claspers which cover portions of the cup beneath. Lateral angles acutely rounded. Ventral border unevenly thickened and deeply, but roundly, notched medianly.

Surface Within Genital-cup. Genital plates present as transparent platelike structures cephalad to the claspers, which are closely adjacent to inner surface of the genital-cup.

The Claspers. Simple, strongly and evenly curved caudad, then laterad, and finally latero-cephalad. Broad at base, tapering irregularly to a sharp inwardly curved darker tip.

The Proctiger. Not attached as far cephalad as that of *Aelia americana* and somewhat deeper within the genital-cup, but with general characters approximating to that of the latter. Dried specimens appear to possess a definite strongly raised median ridge, but, owing to comparatively low degree of sclerotization of this organ in this species, this feature may not exist in the live insect.

Cosmopepla bimaculata

(Thomas, Trans. Ill. St. Ag. Soc., v, p. 455, 1865, *Pentatoma.*) See Fig. 102, 103, 104, 105, 106 and 16.

General Features. Genital-cup opening dorsad, but normally completely retracted beneath seventh tergum of abdomen. Lateral tips acute, and rather closely continuous with the connexivum when segment is completely retracted. Width of genital segment about 1.3 mm.

Borders of Genital-cup. Dorsal border entire and evenly arched. Superior ridge absent. Dorsal or lateral borders not produced over part of the genital-cup as in two preceding species. Lateral angles of cup somewhat indefinite, but acute. Ventral margin smoothly and evenly convex from vertical aspects, and concave from caudal aspect. Inferior ridge present, narrowed medianly and gradually broadening laterad to merge with inner surface of cup near lateral tips of segment. From caudal aspect, inferior ridge appears as deeply and roundly notched medianly.

External Surface of Genital Segment. Visible portion of genital segment somewhat flattened posteriorly. Ventral border somewhat produced as a thinner platelike structure caudad.

Surface Within Genital-cup. Indications of genital plates present latero-cephalad to claspers as small sclerites fused to inner surface of cup (see figure). Hairs numerous on inferior ridge and inner lateral borders.

The Claspers. Simple, erect and curled slightly laterad towards tips. From ental aspect, truncate. From dorsal aspect, bladelike. Shining, smooth and in general small in size compared with those of other species studied.

The Proctiger. Prominent, of even width, except for slight basal constriction and swelling. Of lighter color than the remainder of copulatoria. Horizontal in position and clothed with long caudad directed hairs over basal half.

Cosmopepla conspicillaris

(Dallas, List of Hemip., i, p. 225, 1851, *Nysarcoris.*) See Fig. 107, 108.

General Features. Very similar to those of *C. bimaculata*, the color patterns of the two insects furnishing the easiest method of distinguishing the species. In this species the genital segment is very slightly convex cephalad to the lateral tips, and then shallowly concave, giving it the appearance of being slightly constricted, when viewed from the vertical aspect. In *C. bimaculata* the above outline is shallowly concave, or, at most, straight. In this species the inferior ridge is not evenly notched medianly, as in former species, but with an

irregular depression. Except as above, the descriptive notes for *C. bimaculata* hold true, in general, for this species. (Lack of sufficient material made a detailed study of the aedeagus impossible, but a study of the aedeagus of the

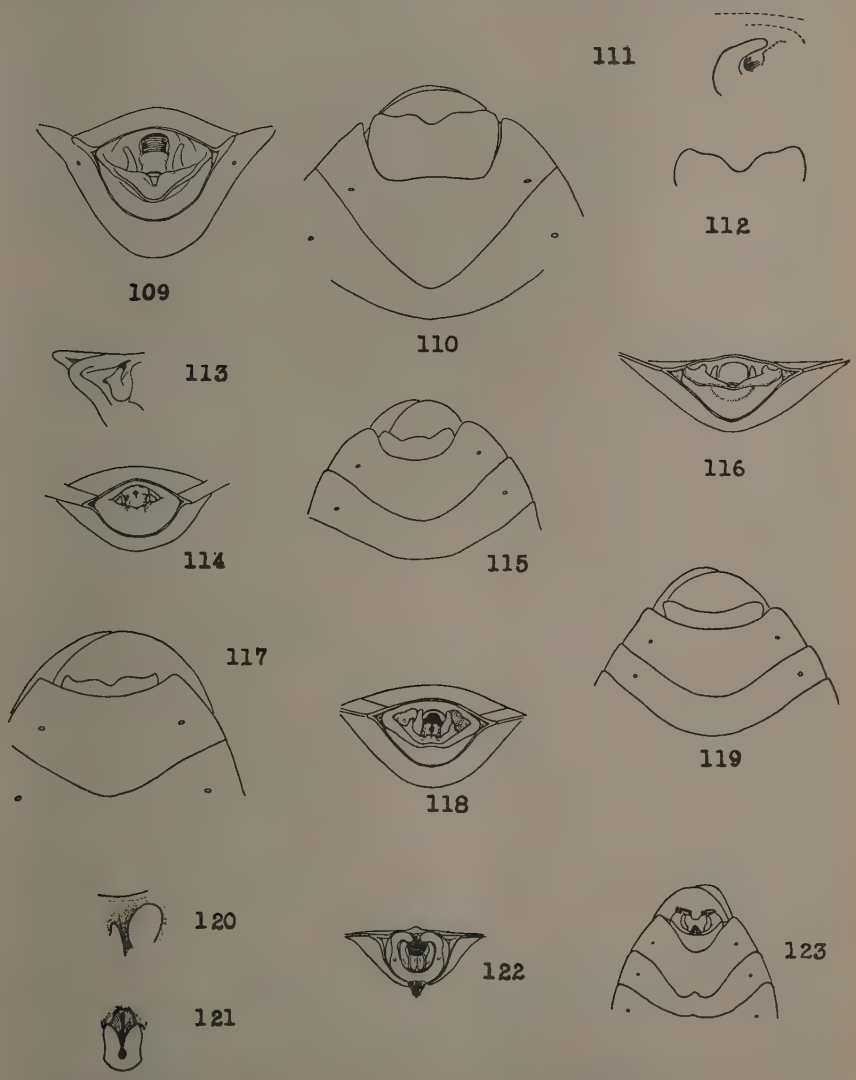


FIG. 109-112. *Menecles incertus Say.* FIG. 109. *Caudal view.* FIG. 110. *Ventral view.* FIG. 111. *Ental view of tip of sinistral clasper in situ.* FIG. 112. *Ventro-caudal view of ventral margin of genital-cup.*
FIG. 113-115. *Thyanta custator Fabr.* FIG. 113. *Latero-caudal view of sinistral clasper, showing relation to genital-cup, etc.* FIG. 114. *Caudal view.* FIG. 115. *Ventral view.*
FIG. 116-117. *Acrosternum hilaris Say.* FIG. 116. *Caudal aspect.* FIG. 117. *Ventral view.*
FIG. 118-121. *Banasa dimidiata Say.* FIG. 118. *Caudal view.* FIG. 119. *Ventral view.* FIG. 120. *Ental view of tip of sinistral clasper, etc.* FIG. 121. *Dorso-caudal view of procliger.*
FIG. 122-123. *Meadorus lateralis Say.* FIG. 122. *Caudal view.* FIG. 123. *Ventral view.*

preceding species was made and the results included under the general remarks on the aedeagus of the Pentatomidae. A description of the aedeagus of this species, when made, can be compared to that of the former and may reveal further specific differences in genital structures.)

Menecles incertus

(Say, Heter. N. Harm., p. 6, 1831; Fitch reprint, p. 762; Compl. Writ., i.p. 317, *Pentatoma*.) See Fig. 109, 110, 111, 112.

General Features. Genital-cup widely open dorso-caudad, but normally covered by wing tips which project beyond. Cup not capable of retraction beneath seventh tergum. Width of genital segment about 2.3 mm. Greatest depth of cup from ventral margin to edge of superior ridge about 1.43 mm. (The latter measurement would be from the caudo-dorsal aspect.) Lip present. Lateral tips squarely rounded.

Borders of Genital-cup. Superior ridge present. Dorsal border evenly rounded and merging mesad into superior ridge. Superior ridge prominent and even in outline except for a shallow median concavity. Lateral angles absent. Ventral border obtusely angled from caudal aspect, (100°), and sinuate to tips.

External Surface of Genital Segment. From lateral aspects, outline of surface below ventral border concave so that the lip appears to overhang somewhat throughout its entire breadth. Ventral surface similar to remainder of abdomen, but usually with an irregular median dark stripe which extends from beneath the seventh sternum caudad, but not as far as ventral border. Hairs absent.

Surface Within Genital-cup. Inferior ridge present and prominent. Ridge cut medianly by a deep abrupt notch. Region of cup caudad to inferior ridge deeply and abruptly depressed. Inner surfaces of lateral borders of cup conspicuously haired; the hairs projecting entad.

The Claspers. Simple, strongly and abruptly curved cephalad. Tips smooth and rounded without sharp terminal narrowing. With fine fringe of cephalic directed hairs on basal cephalic margin. Remainder of organs smooth and polished.

The Proctiger. Basal half of organ smooth, polished and with even transverse grooves. Distal half swollen at point of union with basal half; dull, roughened and clothed with many irregularly arranged fine hairs.

Thyanta custator

(Fabricius, Syst. Rhyng., p. 164, 1803, *Cimex*.) See Fig. 113, 114, 115.

General Features. Opening of genital-cup considerably constricted so that the borders conceal a major portion of the structures within the cup. Width of genital segment about 1.14 mm. Width of genital-cup about 0.7 mm. Opening of genital-cup dorso-caudad. Genital-cup capable of being retracted beneath seventh tergum, but not usually the case. Tips of connexivum normally extend caudad to genital segment. Lip absent. Lateral tips extended and subacute.

Borders of Genital-cup. Dorsal border entire and thickened laterad. Superior

ridge absent. Lateral angles of cup strongly acute (about 45°). Ventral border with slight but not abrupt incisions and semitransparent to near lateral angles.

External Surface of Genital Segment. Ventral surface and lateral tips of segment strongly clothed with conspicuous erect hairs averaging about 0.57 mm. in length. Region below ventral margin denudate adjacent to margin. Dorsal surface of segment denudate. Ventral surface with two slight elevations running from ventral region to lateral tips.

The Claspers. Simple, curve strongly and evenly cephalad to terminate in darker sharp tips. Tips partially concealed in notch in dorsal border of cup. Short broad spur with darkened tip present on mid caudo-lateral region. Organ denudate, smooth and polished.

The Proctiger. Conspicuous, broader than long and without visible transverse striations. With elevated transverse border near base, from which arise a few fine scattered hairs. Remainder of organ denudate and dull. Tip evenly and squarely margined.

Acrosternum hilaris

(Say, Ins. of La., p. 9, 1832; Heter. N. Harm., p. 5, 1831; Fitch reprint, p. 761; Compl. Writ., i, p. 304, 316, *Pentatoma.*) See Fig. 116, 117.

General Features. Genital-cup open broadly dorsad. Genital segment flattened dorso-ventrad, thus conforming to general shape of the abdomen of this insect. Cup capable of complete retraction beneath seventh tergum, but normally only partially so. Width of genital segment about 1.86 mm. Width of aperture in seventh segment about 2.14 mm. With a paired lip. Lateral tips never continuous with connexivum and acute.

Borders of Genital-cup. Dorsal border normally completely concealed beneath the seventh tergum when segment is retracted. Superior ridge present and interrupting dorsal border. Lateral angles of cup acute and crenulate. Ventral border with rounded median notch and sinuate to tips. Ventral border broadens considerably laterad, from where four dorsally directed elevations arise, each pair being connected by a slight oblique ridge. Inner projections immediately laterad to claspers. Outer projections form the elevated portion of the lateral tips. Inferior ridge absent.

External Surface of Genital Segment. Region below ventral border strongly creased in the form figured. Segment lighter in color and denudate.

The Claspers. Simple; directed dorsad, bladelike, tapering abruptly at tip, lighter in color, tips denudate, and polished.

The Proctiger. Prominent. as broad as long (0.57mm.). Not banded, but smoothly punctate and clothed with inconspicuous minute sparse hairs. Transversely elevated near base and broadening slightly to tip, which is squarely entire and somewhat shining.

Banasa dimidiata

(Say, Heter. N. Harm., p. 7, 1831; Fitch reprint, p. 763; Compl. Writ., i, p. 318, *Pentatoma.*) See Fig. 118, 119, 120, 121.

General Features. Genital-cup broadly open to dorso-posterior, but nor-

mally hidden by wing tips. Width of genital segment about 1.7 mm. Dorsal region of cup normally slightly retracted under seventh tergum. Lateral tips acutely rounded. Lip absent.

Borders of Genital-cup. Dorsal border interrupted by superior ridge, which projects prominently over base of proctiger. Superior ridge cut medianly by a deep U-shaped incision. Dorso-lateral borders evenly concave. Ventral border thickened and evenly concave from vertical aspects. From caudal aspect, ventral border with a slight median flattening. Lateral angles of cup acute. Dorso-lateral borders, ventral margin, and lateral angles with many fine hairs projecting towards centre of genital-cup.

External Surface of Genital Segment. Somewhat flattened below ventral border of cup. Flattened area bald and with larger punctation than remainder of abdomen. Segment scarlet.

Surface Within Genital-cup. Inferior ridge present and prominent, ending laterally in two conspicuous, dorsally directed spines. Median region of inferior ridge with two slight elevations.

The Claspers. Simple, directed strongly dorsad and laterally flattened. Clothed with fine pile. Not sharply differentiated in texture or color from remainder of genital structures. Not shining. Spurs or tubercles absent.

The Proctiger. Not prominent. About 0.43 mm. long by about 0.3 mm. in width. Basal portion constricted where it passes under superior ridge. Organ grooved dorsally along median line to terminate in a small pitlike depression a little distance from tip. Distal portion swollen, pilose, and not polished, but of same color and general texture as remainder of genitalia.

Meadorus lateralis

(Say, Heter. N. Harm., p. 3, 1831; Fitch reprint, p. 757; Compl. Writ., i, p. 312, *Edessa.*) See Fig. 122, 123, 124, 125.

General Features. A species whose male genital characters are widely different from any other species of Pentatomidae occurring in our Canadian fauna, making it extremely easy of identification in this way. Genital-cup not visible dorsad, but widely open ventro-caudad. (Two specimens were obtained with the genital segment retracted in an inverted position, with the result that the genital-cup appeared to open dorso-caudad.) Opening of genital-cup never concealed, although segment may be almost completely retracted to the level of the caudal borders of the seventh segment. Width of genital segment about 1.14 mm. Vertical measurement of segment about 0.86 mm. Genital-cup comparatively shallow, so that the contained structures are plainly visible. Lip absent. Lateral tips absent, but lateral margins slightly acute from vertical aspects.

Borders of Genital-cup. Dorsal border deeply and roundly cleft medianly, and with lateral portions conspicuously thickened from cleft and evenly arched. Lateral angles of cup absent. Ventral border with a converging tuft of aggregated hairs. Dorsal border capillate, the tips of the hairs being curled entad. Dorsal tufts and ventral tuft creber and erect. Latter matted at tips and projecting ventro-caudad.

External Surface of Genital Segment. Denudate and polished. Strongly and evenly convex.

Surface Within Genital-cup. Two small lamellae present within lateral border of cup, the bases of which fuse dorsally with the interior surface of cup. These are probably the much reduced genital plates and would be hardly

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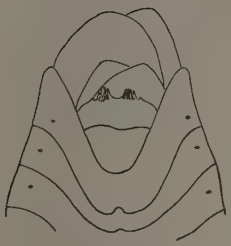
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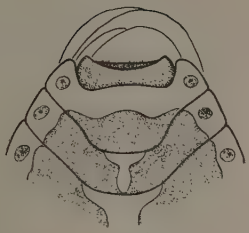
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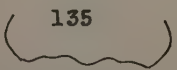
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FIG. 124-125. *Meadorus lateralis Say.* FIG. 124. *Dorsal view showing the relations of the genital segment (9th) to the 7th tergum.* FIG. 125. *Ental view of sinistral clasper in situ.*

FIG. 126-129. *Elasmostethus cruciatus Say.* FIG. 126. *Details of inferior ridge and bases of claspers.* FIG. 127. *Outline, from lateral aspect, of extremity of abdomen.* FIG. 128. *Caudal view.* FIG. 129. *Ventral view.*

FIG. 130-132. *Elasmostethus atricornis Van D.* Fig. 130. *Caudal view.* FIG. 131. *Ventral view.* FIG. 132. *Caudal view of sinistral clasper.*

FIG. 133-135. *Perillus bioculatus Fabr.* FIG. 133. *Ventral aspect.* FIG. 134. *Subcaudal view of sinistral half of genital-cup showing details of clasper and genital plate, etc.* FIG. 135. *Caudal aspect of ventral margin of genital-cup.*

FIG. 136-138. *Perillus circumcinctus Stal.* FIG. 136. *Caudal view.* FIG. 137. *Caudal view of ventral border of genital-cup.* FIG. 138. *Subcaudal view of sinistral half of genital-cup showing details of clasper, genital plate, etc.*

noticeable if it were not for the small tips projecting ventro-caudad.

The Claspers. Simple, erect and projecting dorso-caudad. Unevenly ridged longitudinally, presenting the appearance of slight spiral twisting. Tips rounded and as broad as base. No conspicuous projection. Denudate and polished with tips slightly darker.

The Proctiger. Small, vertical, and posteriorly flattened, with raised lateral and terminal margins. From median region, terminal margin produced caudad into a sharp spine. Organ pilose. Region above organ vaulted and transversely ridged.

Elasmostethus cruciatus

(Say, Heter. N. Harm., p. 2, 1831; Fitch reprint, p. 756; Compl. Writ., i, p. 311, *Edessa.*) See Fig. 126, 127, 128, 129.

General Features. Genital-cup shallow and widely open caudad. Sternum of eighth segment plainly visible. Lateral tips of connexivum normally project strongly caudad to ventral margin of genital segment. Lateral tips absent and sides never closely adjacent to connexivum. Width of segment about 1.57 mm. and vertical measurement about 1.14 mm. Segment capable of being completely retracted beneath seventh tergum, but not the sternum. Genital plates absent.

Borders of Genital-cup. Borders evenly rounded, without lateral angles or any irregular depressions. Ventral border produced farther caudad than dorsal border and with two tufts of converging aggregated hairs which project strongly caudad. Laterad to these tufts are two short sharp spines. Remainder of borders denudate and smooth.

Surface Within Genital-cup. Inferior ridge present, immaculate and as figured in detail.

External Surface of Genital Segment. Of same general texture, color, etc. as remainder of abdomen and without any easily discernible markings or depressions.

The Claspers. Simple, erect and with extremities connivent over base of proctiger. Denudate, polished and flattened cephalo-caudad. Round and broader at tips, with sharp lateral spur.

The Proctiger. Of same texture as remainder of genital segment. Widest at base and tapering to tip, which is produced somewhat caudad.

Elasmostethus atricornis

(Van Duzee, Trans. Am. Ent. Soc., XXX, p. 75, 1904, *Acanthosoma.*) See Fig. 130, 131, 132.

General Features. Genital-cup widely open to the posterior and contained structures always visible. Eighth sternum plainly visible. Connexivum tips project strongly caudad to genital segment. Lateral tips present, but broadly and obtusely rounded, never closely adjacent to connexivum. Width of genital segment about 1.43 mm. Segment capable of retraction beneath seventh tergum, but not the sternum.

Borders of Genital-cup. Dorsal border entire and evenly arched to lateral angles. Lateral angles obtusely rounded. Ventral border produced farther

caudad than dorsal border and with two tufts of convergent aggregated hairs which project caudad. Hair tufts not as large or conspicuous as those of *E. cruciatus*, and without spines laterad to them.

External Surface of Genital Segment. Similar to remainder of abdomen.

The Claspers. Simple, erect and with tips connivent over base of proctiger. Flattened cephalo-caudad, denudate, tips broader than base and extending laterad. Sharp lateral spur absent. Outline from caudal aspect specific, rendering the species easily distinguishable from *E. cruciatus*.

The Proctiger. Of same general outline and texture as that of preceding species but with indications of superior ridge over base.

Perillus bioculatus

(Fabricius, Syst. Ent., p. 715, *Cimex*.) See Fig. 6, 133, 134, 135, and 17.

General Features. Genital-cup open completely caudad and partially hidden by wing tips normally. Width of segment about 1.57 mm. Vertical caudal measurement of visible portion of segment 1.3 mm. of which only about 0.43 mm. is of the genital-cup. Eighth segment not normally visible. Lateral tips present, but broadly rounded and normally projecting but little caudad of connexivum tips. Genital-cup usually slightly retracted beneath seventh tergum. Lip absent and ventral border vertical.

Borders of Genital-cup. Dorsal border triconcave, with the median concavity arching the base of the proctiger. Superior ridge absent. Lateral angles of cup widely and obtusely rounded, with sudden thickenings of borders near tips which bear prominent erect hairs projecting mesad from inner surface.

External Surface of Genital Segment. Region below ventral margin differentiated from remainder of external surface. This region with a round median depression and oblique lateral grooves, denser and more irregular punctation (similar to that of proctiger), and longer, sparse, scattered hairs. Genital segment black, with lighter reddish tinge in region of lateral tips, punctate, without vestiture, and sharply differentiated from seventh segment by the difference in color.

Surface Within Genital-Cup. Genital plates present and prominent, being situated cephalad to claspers. Genital plates squarely broad reaching to dorsal margin, crenulate on their dorsal and mesal margins, and considerably lighter in color than any adjacent structure.

The Claspers. Simple, strongly and broadly eflected. From caudal aspect, triangular; being broadest at base. Prominently ridged entad and ventrad. Dorsal margin not ridged and evenly concave from caudal aspect; ventral outline, from same aspect, evenly convex. Tip acutely rounded. Greatest horizontal measurement of deflected portion of organ about 0.57 mm. Greatest vertical measurement about 0.43 mm. Smooth and shining.

The Proctiger. Prominent and of uniform texture. Broadest at base, with slightly raised median area to tip. Tip broad and evenly rounded. Of same texture, color, etc. as region below ventral border of genital-cup, but without vestiture of any kind.

Perillus circumcinctus

(Stal, Stet. Ent. Zeit., XXIII, p. 89, 1862.) See Fig. 136, 137, 138.

General Features. Similar in general appearance to *P. bioculatus*, but with many definite specific characters. Genital-cup open incompletely caudad and partially hidden by wing tips normally. Width of genital segment about 1.43 mm. Vertical measurement of visible caudal portion of genital segment about 1 mm. of which about 0.57 mm. is of the genital-cup, (*i.e.*, the genital-cup is open more widely caudad than that of *P. bioculatus*). Lateral tips inconspicuous, more so than those of preceding species of this genus. Hairs on tips dorsally directed. Genital-cup usually slightly retracted beneath seventh tergum. Lip absent and ventral border subvertical.

Borders of Genital-cup. Dorsal border similar to *P. bioculatus*, but with lateral angles more broadly rounded. Superior ridge absent. Lateral angles widely and obtusely rounded, with borders suddenly thickened near tips. With prominent erect hairs projecting mesad from inner surface of cup near lateral angles.

External Surface of Genital Segment. Region below ventral margin of cup differentiated, with deep but broad median depression, and with longer scattered hairs. Genital segment brownish, with lighter shades in regions of lateral tips and below ventral margin. Sharply differentiated from seventh segment by darker color.

Surface Within Genital-cup. Genital plates present and prominent. More rounded dorsad than those of preceding species of this genus and not so conspicuously crenulate. Light in color and without vestiture.

The Claspers. Simple, strongly and broadly eflected. From caudal aspect, sharply angulate at inner dorsal angle. Dorsal margin slightly convex, never concave. Prominently ridged entad, but to a lesser degree ventrad than *P. bioculatus*. Tip more broadly rounded than preceding. Horizontal measurement of deflected portion about 0.5 mm. Greatest vertical measurement about 0.35 mm. Smooth and shining.

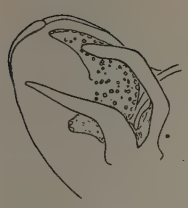
The Proctiger. Prominent and of uniform texture. Similar to preceding, but more strongly ridged medianly and dark brown in color.

Apateticus bracteatus

(Fitch, 3d. Rept., Trans. N.Y. St. Agr. Soc., XVI, p. 336, 1856, *Arma.*) See Fig. 139, 140 and 15.

General Features. Generic genital characters very distinct. Genital-cup shallow, opening caudad and somewhat ventrad. Proctiger arises dorsad of cup proper and the latter forms two earlike lobes laterad to the point of junction of the former. Width of genital segment about 2.7 mm. Lateral tips present, but rounded and extending slightly dorsad to region of connexivum, with which they are usually adjacent, but not contiguous. Without any prominent ventral border other than a transverse ridging. But little of external surface of genital segment normally visible when retracted.

Borders of Genital-cup. Superior ridge absent. Dorsal border evenly arched over base of proctiger and then extending strongly dorsad, concavate, to



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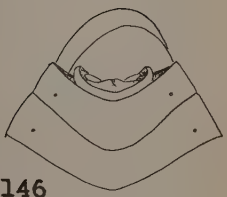

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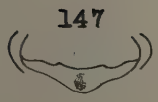

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FIG. 139-140. *Apateticus bracteatus Fitch.* FIG. 139. *Caudal view of sinistral half of genital-cup showing details of clasper, genital plate, etc.* FIG. 140. *Caudal view.*

FIG. 141-143. *Podisus maculiventris Say.* FIG. 141. *Caudal view showing detail of ventral and lateral borders of genital-cup, superior ridge, etc.* FIG. 142. *Latero-caudal view of sinistral half of genital-cup showing details of clasper, genital plate, etc.* FIG. 143. *Caudal view.*

FIG. 144-146. *Podisus serieventris Uhl.* FIG. 144. *Details of claspers, genital plates, proctiger, and ventral and lateral borders of genital-cup, from the caudal aspect.* FIG. 145. *Caudal view.* FIG. 146. *Ventral view.*

FIG. 147-149. *Podisus modestus Dall.* FIG. 147. *Caudal view showing details of ventral and lateral borders of genital-cup.* FIG. 148. *Caudal view.* FIG. 149. *Latero-caudal view of sinistral half of genital-cup showing clasper, genital plate, etc.*

FIG. 150-152. *Podisus placidus Uhl.* FIG. 150. *Caudal view showing details of ventral and lateral borders of genital-cup.* FIG. 151. *Caudal view.* FIG. 152. *Latero-caudal view of sinistral half of genital-cup showing clasper, genital plate, etc.*

FIG. 153-155. *Zicrona caerulea Linn.* FIG. 153. *Dorsal view (wing tips removed).* FIG. 154. *Caudal view.* FIG. 155. *Latero-dorsal view of sinistral clasper in situ.*

lateral tips. Lateral and ventral borders continuous and evenly concave, becoming less strongly marked ventrad. With long (0.3 mm.) hairs (not dense) projecting mesad from inner surface of lateral borders. Ventral border bearded, in median area, with long ventrally projecting hairs which converge to two submedian points.

Surface Within Genital-cup. Genital plates present and prominent, having the appearance of large tuberculate earlike plates, cephalad to the claspers, with their midectal margin merging into the inner surface of the genital-cup. Inferior ridge present, prominently projecting caudad, and clothed with long aggregated hairs which curl caudo-mesad so that the tips intermingle closely.

The Claspers. Bidactylate, the bifurcation occurring near base of organ. Dorsal process extends dorsad to curve laterad near its tip, so as to follow the lateral portions of the dorsal border of the genital-cup. Ventral process caudad to that of the dorsal process at base and of approximately the same length and shape, but curving laterad close to its point of origin. Organs destitute of vestiture, smooth, shining and tapering gradually to acutely rounded points which project laterad and somewhat caudad.

The Proctiger. Vertical, conspicuous, but not normally raised above level of genital-cup. Narrower towards base, clothed with vestiture of fine hairs, of same color and texture as genital segment and with a prominent median boss on basal portion.

Podisus maculiventris

(Say, Ins. of La., p. 11, 1832, *Pentatoma;* reprint in Psyche VIII, p. 307, 1899.) See Fig. 141, 142, 143.

General Features. Presenting many general characters that are common to *Perillus* spp. Genital-cup opening caudad, but with dorsal border retracted normally beneath seventh tergum so that only lateral tips are usually visible from above. Width of genital segment about 1.5 mm. Lateral tips present, but broadly rounded and normally projecting but little. Ventral border rising dorso-caudad. Genital-cup shallow, so that claspers and genital plates are visible from above when segment is extended.

Borders of Genital-cup. Dorsal border evenly arched over base of proctiger with an inconspicuous bilobed superior ridge as a caudal extension. From proctiger, dorsal border arches dorso-laterad to lateral angles of cup, which are broadly rounded. Outline of ventral margin as figured in detail.

External Surface of Genital Segment. Region below ventral margin of genital-cup differentiated from remainder of genital segment, and with median portion swelling caudad. Median swelling bearing two adjacent tufts of stout, erect, lighter colored hairs, which curl slightly mesad. Ventral margin finely haired. Tips and ventral regions of segment with many conspicuous hairs, those in the latter region being longer.

Surface Within Genital-cup. Genital plates present and conspicuous but not visible laterad. These organs tuberculate, with thicker dorsal borders curling slightly caudad, giving them the appearance of being free appendages.

The Claspers. Simple, strongly and broadly eflected. Explanate region

triangular from caudal aspect. From latter aspect, ental margin ridged, ventral margin convex, and dorsal margin concave. Dorso-basal region projects slightly dorsad beyond the limits of the genital-cup. Horizontal measurement of deflected portion of organ about 0.35 mm. and greatest vertical measurement also around 0.35 mm. Destitute of vestiture and shining.

The Proctiger. Position subvertical. Prominent and of uniform texture. Capillate.

Podisus serieventris

(Uhler, Proc. Bost. Soc. Nat. Hist., XIV, p. 94, 1871; XIX, p. 370, 1878.) See Fig. 144, 145, 146.

General Features. Showing very close relationship to preceding species. Width of genital segment about 1.43 mm. Other general characters as for *P. maculiventris.*

Borders of Genital-cup. Dorsal border but slightly arched over base of proctiger. Superior ridge absent. Lateral portions of dorsal border arching but little dorsad. Ventral margin with less abrupt depressions than preceding species.

External Surface of Genital Segment. Surface similar to *P. maculiventris,* including location and size of hairs.

Surface Within Genital-cup. Genital plates present and similar to preceding species, but slightly broader.

The Claspers. Simple, strongly and broadly eflected. Explanate region triangular and, from caudal aspect, with dorsal margin shallowly concave, ventral margin convex, and ental margin not ridged. Dorsal angles of organs do not project dorsad to the genital-cup. Size the same as for preceding species. Destitute and shining.

The Proctiger. Prominent and of uniform texture. Vertical, and of same color and composition as genital segment. Capillate, and with two basal swellings from which proceed two indefinite ridges which converge toward the tip.

Podisus modestus

(Dallas, List of Hemip., i, p. 101, 1851, *Arma.*) See Fig. 147, 148, 149.

General Features. As for two preceding species. Width of genital segment about 1.14 mm.

Borders of Genital-cup. Dorsal border evenly arched over base of proctiger and evenly concave laterad to region of lateral angles. Superior ridge absent. Outline of ventral border as figured, with depressions as abrupt as those of *P. maculiventris,* and more so than *P. serieventris.* Median portion of ventral border arched more strongly caudad than two preceding species.

External Surface of Genital Segment. Region below ventral margin not as sharply differentiated from remainder of segment as in two preceding species. External surface haired, but not conspicuously aggregated in any region. (Some specimens only sparsely haired.)

Surface Within Genital-cup. Genital plates present, and similar to those of other members of this genus except for smaller size (in proportion to segment).

These organs with more irregular borders and projecting slightly in two small lobes latero-ventrad to the claspers.

The Claspers. Simple, strongly and broadly eflected. Explanate region triangular from caudal aspect, with dorsal margin conspicuously concave, ventral margin convex, ental margin not ridged, and with dorsal angles produced but not extending dorsad of genital-cup. Smooth, denudate and polished. Horizontal measurement of deflected portion about 0.3 mm. and greatest vertical measurement also 0.3 mm.

The Proctiger. Of uniform texture, and vestiture similar to preceding species of this genus, but with faint median ridging.

Podisus placidus

(Uhler, Am. Ent., ii, p. 203, 1870.) See Fig. 150, 151, 152.

General Features. In general, consistent with remainder of genus, but with distinct specific features that render the species easily distinguishable. Width of genital segment about 1.35 mm.

Borders of Genital-cup. Dorsal border extending farther cephalad than other species of this genus. Superior ridge absent. Dorsal border entire over proctiger and sinuate to lateral angles of genital-cup. Outline of ventral margin appears specific.

External Surface of Genital Segment. Region below ventral border of genital-cup as figured, and destitute of vestiture.

Surface Within Genital-cup. Genital plates present and prominent. These organs, with dorsal borders, extending caudad so as to overhang slightly above the claspers.

The Claspers. With general features of preceding species of this genus, but with a dorsal concavity on explanate portion so strongly accentuated that the organs might more correctly be termed bifurcate. The outlines, as seen by figurations, are quite distinct.

The Proctiger. Surface even and of uniform texture, and vestiture similar to other species of this genus.

Zicrona caerulea

(Linnaeus, Syst. Nat., Edn. 10, i, p. 445, 1758, *Cimex.*) See Fig. 153, 154, 155.

General Features. A species with genital characters that admit of no confusion with any of the species already described. Opening of genital-cup dorsad, and normally completely covered by wing tips. Genital-cup never retracted beneath seventh tergum. Lateral tips of genital segment, when retracted, closely contiguous with connexivum to form a continuous, even border for the abdomen. Eighth segment not normally visible.

Borders of Genital-cup. When wing tips are removed, dorsal border always completely visible. Surface of genital segment, cephalad to dorsal border, ridged medianly, denudate and shining. Lateral angles of genital-cup absent, the lateral borders merging into inner lateral surface of ventral border. With conspicuous, short erect hairs on inner surface of lateral borders, becoming sparser within ventral border. Ventral border evenly arched caudad.

Superior ridge, inferior ridge, and genital plates, absent.

The Claspers. Simple, bladelike, mucronate and falciform, with tips normally extending well beyond the genital-cup, as figured. Basal regions touching each other caudad to tip of proctiger, from there they curve latero-cephalad. Polished and destitute of vestiture.

The Proctiger. Horizontal in position and much abbreviated. Not elevated, and but slightly depressed below the level of the borders of the genital-cup. Of even texture throughout visible area. With hairing directed caudad on margin of tip. Remainder of organ denudate and polished.

X. Suggested Line of Development of the Male Genital Structures of the Pentatomidae

Kirkaldy believed that the Pentatomidae were the most primitive Hemiptera (Heteroptera). Hart (1919) agreed with this view, and claimed that the five-segmented antennae of these insects were more primitive than the four-segmented antennae of other families of bugs and, although the antennae of pentatomid nymphs are uniformly four-segmented, he did not think the antennae of the adult the result of specialization. If this view is the correct one, the study of the possible evolution of the male genitalia of these insects should throw considerable light on the same organs of other groups of Hemiptera.

It has been maintained by some specialists of insect anatomy that the abdomen of an insect is primitively composed of twelve segments. The question as to whether or not the primitive insect abdomen consisted of eleven segments and a telson, or of a lesser number, cannot be answered by a study of the group under consideration. In the Pentatomidae there are never more than ten apparent segments to the abdomen in any stage. The possibility of the existence of a rudimentary eleventh segment is referred to.

In that division of this paper containing a general discussion of the male organs in insects in relation to this group, the writer has given an outline of the possible homology of the terminal structures of the abdomen, which will serve as an introduction to the tentative suggestions that follow.

The abdomens of the Pentatomidae are campanulate. That is, the segments are convex on their cephalic margins and concave on their caudal margins. The convex cephalic end of one segment fits into the concave caudal margin of the preceding segment. The concavity in the caudal end of the next to last segment (apparent), is filled by the apparent caudal segment, in the adult males, and this latter segment (ninth) has its caudal margin convex, or nearly so. This arrangement of the segments is usually more strongly marked on the ventral than on the dorsal side of the abdomen.

In the study of the male genitalia we are not immediately concerned with the abdominal segments cephalad to the eighth segment, and in the primitive condition I assume that at least segments nine and ten bore appendages. The male genital opening has been located between the ninth and tenth sterna in the membrane connecting the two, thus involving these two segments in the essential structures of the genitalia in addition to any further modifications

that may occur in the eighth. While the homology of the limits of the ninth and tenth segments is not always possible, it has been indicated that the armature surrounding the genital opening are developments of the ninth abdominal segment, and that those structures surrounding the anal opening are usually developments of the tenth.

In the nymphs of the Pentatomidae the eighth and ninth segments are visible only as circular rings and the tenth segment projects slightly to the rear of these again. This is the condition I have figured graphically in *a*, Fig. 22. The close association of the anal opening and the eleventh segment in more generalized insects, would indicate the possibility that an additional terminal segment might be represented by the slightly sclerotized borders surrounding the anus.

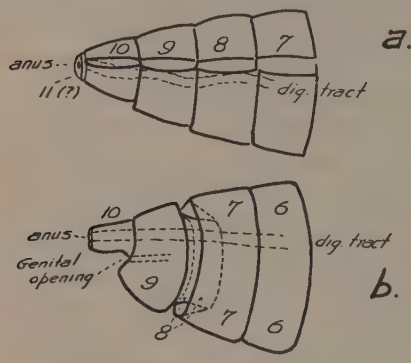

FIG. 22. *Diagrammatic drawings illustrating the suggested line of development of the terminal segments of the abdomen in the Pentatomidae. a, nymph; b, hypothetical condition between last instar and adult.*

In the later instars of the male Pentatomidae sexual differences are sometimes noticeable, due to the growth of the penultimate or ninth segment. This segment becomes swollen on the ventral side, where it is often larger at the middle than near the sides and, in the final nymphal stage, a transverse depression may appear in this swollen region so that portions of it come to lie ventrad of the tenth sternum. These changes do not occur in the female.

Along with the above changes we find a corresponding reduction in size (comparative) in the segments immediately cephalad and caudad to the ninth. Growth of the eighth segment is restricted until it appears as a narrow ring and, in the final stage resulting in the adult, it becomes invaginated or telescoped into the seventh segment. The comparative size of the tenth segment becomes reduced, both laterally and dorso-ventrally and, with the swelling of the sternum of the ninth segment, its position tends to move dorsad. In *b*, Fig. 22, this evolution is depictured, and indicates a hypothetical condition as it might exist between the last nymphal stage and the adult, rather than that of either of these stages. The changes in the penultimate segment have been noted by authors (*e.g.* Hart, 1919) and Walker (1919) has recorded a similar phenomenon as occurring in the later immature stages of the males of *Grylloblatta campodeiformis*.

In the adult condition the changes described above are further accentuated. The depression in the ventro-caudal region of the ninth segment has proceeded cephalad; tending to carry the greatly reduced tenth segment forward into the cavity thus formed. The eighth segment has completely, or almost completely, disappeared within the segments anterior to it. Segments six and

seven remain in evidence with the anterior borders of the ninth or genital segment serving to fill the crescentic depression occasioned by the concave caudal margin of the seventh segment.

From the description of the various structures found in males of this group, which have already been given, it becomes evident that the genital-cup has been formed through the sternal depression of the ninth segment. This invagination has resulted in the ventro-caudal margin of the ninth segment coming to lie deeply within the formed genital-cup and beneath the greatly reduced flaplike tenth segment or proctiger. Secondary depressions ventrad of the primary depression in the ninth segment are evident in several species.

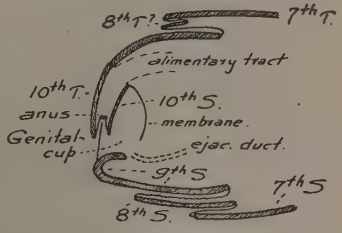

FIG. 23. *Diagrammatic drawing illustrating subsequent development of terminal segments from that shown in Fig. 22. Genital-cup opening directly to the rear.*

The aedeagus is located beneath the proctiger, or what is here homologized as being the remains of the tenth segment. If the homologies suggested above are correct, the genital opening occurs in the membrane connecting the ninth and tenth sterna. This is presumed to be the primitive position of that organ. Located on either side and partly beneath the aedeagus are the claspers, which may possibly represent rudiments of the appendages of the ninth segment.

This structure herein referred to as the "genital or ninth segment" is probably derived from the ninth sternum.

It seems improbable that the tergum of the ninth segment has entered into the formation of the proctiger, although I recognize that this may have occurred in other orders. In the Pentatomidae I have not been able to see any evidence that would justify me in assuming that any part of the proctiger is derived from the ninth segment. That this condition in the Pentatomidae is apparently not confined to this group is evident from the findings of workers in other orders, *e.g.* Busck and Heinrich (1921) in describing the tegumen of the Microlepidoptera state: "..... but the structure is normally so fused that it is impossible to differentiate two distinct parts, and we are inclined to consider the entire chitinized tegumen as part of the 10th segment; the 9th segment being greatly reduced and continues dorsally as membrane only."

The structures herein termed the genital plates, which are found in many species of this family, are evidently outgrowths of the ninth segment. If the genital-cup were to be evaginated back to its more primitive condition, these organs would exist as lateral, platelike prolongations of the ninth sternum, with their free edges extending dorsad, caudad and ventrad. It is possible that the presence of these plates may represent a more primitive condition than when they are absent. In the latter case it could be assumed that atrophy or fusion had occurred. The relationships between the claspers and these plates appear interesting and should be studied further.

If the above findings are correct, the most primitive type of male genitalia in the Pentatomidae might be one where the formation of the genital-cup had

not proceeded very deeply, and where it opens directly to the rear. A deep genital-cup opening dorsad would be more highly specialized. A proctiger which is capable of retraction within the genital segment would be more specialized than one where such movement was not possible. Organization for dorsal flexion of the proctiger indicates a greater degree of specialization than where such articulation is not possible, and may be described as a separate line of development from where retraction of the structure is possible, neither one being evolved from the other, but both from a proctiger where neither movement was possible. Retractive powers and ability for dorsal flexion of the proctiger are usually accompanied by a caudo-dorsal development of the ventral border of the genital-cup, which has already been referred to as a specialized condition.

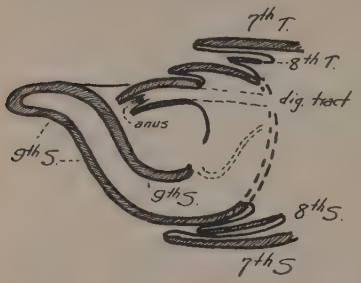

FIG. 24. *Diagrammatic drawing illustrating further development of terminal segments from that shown in Fig. 23.*

While the organs visible externally have been emphasized in my comparative studies, there appears to be some confirmation for the above suggestions in the study of the aedeagus. The presence of a penisfilum appears to represent a somewhat specialized condition. In such species we frequently find a genital-cup which opens chiefly dorsad and often with a prominent liplike structure, the presence of an inferior ridge, a differentiated proctiger with an accompanying development of a superior ridge, and the absence of genital plates. In those species where a penisfilum is absent the genital-cup is more likely to be more shallowly invaginated and to open directly to the rear, the proctiger to present an undifferentiated surface (may even arise slightly dorsad of genital-cup), the genital plates present, superior and inferior ridge may be absent, and the eighth segment may not be entirely concealed *in situ*.

Separate lines of development of the various genital structures are very evident, but a study of many more forms than those included in this paper would be necessary in order to fill many gaps in the stages of specialization. The claspers of one species cannot in general be described as being more primitive than those of another, as there appear to be several separate lines of specialization along which these organs have developed. The vestiture of the genital structures, projections within the genital-cup, spines, secondary infoldings, presence or absence of lateral tips to the genital segment, etc., would appear to all come under this general heading.

The formation of a superior ridge has apparently been coincident with the specialization of the mobility of the proctiger. It is considered as a specialized condition. The "secondary" infoldings which occur in some species below the ventral margin of the genital-cup proper are probably carried deeper and deeper until that portion of the genital-cup, which might previously have been termed the ventral margin, becomes little more than a transverse ridge lying within the genital-cup, protecting and concealing the tip of the proctiger.

In such cases the ventral border of the secondary depression becomes the ventral border, and the former margin goes to the formation of that structure herein termed the inferior ridge. If this is the case, a genital-cup without these secondary depressions or without an inferior ridge would be more primitive in this regard than where either or both these features were present. In the same way, the presence of an inferior ridge would appear to be a more specialized condition than where only a secondary depression occurs. In some species the line of demarcation is very fine between the conditions where a secondary depression exists beneath the ventral margin of the genital-cup, and where what is called an inferior ridge is present within the ventral margin. The development of the inferior ridge through such a secondary invagination of the genital segment appears to be quite evident in some cases.

The structure and various modifications of the aedeagus have been dealt with fairly thoroughly in this paper already. From the foregoing my views of the relationships of these parts should be made more clear. The aedeagus itself varies in shape in the different species, but the essential basic plan is apparent throughout all the forms examined. The basal plate, composed of two lateral sclerotized wings with a connecting bridge and a median projection, is always to be found and in all cases examined is connected to the infolded primitive posterior margin of the ninth sternum, through its ventral connectives. This is the most marked connection that the aedeagal structures have with any of the other sclerotized structures. With the structures at rest the tendency is for the aedeagus to fold cephalad over its basal plate, the point of articulation being the aedeagal hinge. Where the genital-cup opens directly to the rear of the insect the aedeagus proper often points subdorsad, but where the genital-cup opens more dorsad the tendency is for the aedeagus, from its hinge, to be pressed more cephalad beneath the proctiger. While the basic plan of the genitalia is traceable throughout the family there is a wide range of variation and several distinct types are indicated. Further study of the male genitalia of closely related families, such as the Scutelleridae, is necessary before really conclusive statements can be made on the question of relationships.

XI. Summary

1. The development of our knowledge of the male genitalia of the Pentatomidae is indicated and the more important papers reviewed in the light of the writer's findings.

2. Specialization of the eighth, ninth, tenth and, possibly, the eleventh segments, is associated with the reproductive functions.

3. The eighth abdominal segment is always present, although much reduced in size and, normally, partially or completely retracted within the abdominal segments anterior to it. It bears no appendages.

4. The intersegmental membranes cephalad and caudad to the eighth segment are considerably developed. This allows for the eversion of the genital segment (ninth), and permits a great deal of mobility for the copulatory organs.

5. The ninth segment is specialized for the accommodation of the genital structures. This is accomplished by the invagination of its posterior margins

to form a bowl-like depression or genital-cup, within which the copulatory organs lie.

6. The claspers are homologized as originating from the primitive latero-caudal borders of the ninth sternum.

7. The aedeagus is homologized as originating from between the ninth and tenth sterna. It is connected to the ninth sternum.

8. The genital plates are present in many species, chiefly among the more primitive forms. They are homologized as originating from the primitive lateral walls of the ninth sternum.

9. The tenth segment is much reduced in size, existing as a small movable tail-like structure within the genital-cup. The upper surface is usually heavily sclerotized and often ornamented. The under surface is unexposed *in situ* and more membranous than the upper surface. This segment normally conceals and protects the aedeagus.

10. The eleventh segment is not apparent, but there is a possibility that it may exist in the slightly sclerotized folds found bordering the caudal extremity of the tenth segment.

11. The anus opens at the tip of the abdomen, *i.e.*, from the end of the proctiger, which is homologized as the tenth and, possibly, the eleventh abdominal segment.

12. The aedeagus varies considerably in form, but the basic plan is clearly indicated.

13. The basal plate of the aedeagus is made up of four sclerotized regions and two pairs of connectives, a ventral pair of connectives running to the infolded margin of the ninth sternum and a dorsal pair running dorso-cephalad.

14. The aedeagus is articulated with its basal plate and continues distad as a sclerotized cylindrical structure, the penial theca, which encloses a large seminal reservoir.

15. At the distal end of the penial theca the more thinly sclerotized penis is located. The penis may be two- or three-lobed.

16. The ejaculatory duct has been traced from its free end to an opening in the median projecting sclerotization of the basal plate of the aedeagus. It is joined by a duct from the seminal reservoir in the penial theca.

17. The ejaculatory duct terminates distad between the two lateral penis lobes. When a median penis lobe is present the duct passes through this structure. In some species a conspicuous penisfilum is formed.

18. A distinct connection between the clasper and the ninth sternum is present in certain species, which is independent of the aedeagal connections.

19. The genital structures of the male and female are in their correct relation to each other when the insects assume the position of posterior to posterior. Owing to the great mobility of the genital segment this is not a necessary, nor the usual, initial position adopted during copulation.

20. The functions of the claspers are apparently twofold; to assist in separating the genital sclerites of the female, and to assist as clasping organs during copulation.

21. The aedeagus is capable of some extension beyond the limits of the genital-cup in addition to that brought about by the extension of the penis lobes. The movements of the basal plate and its connectives are described.

22. In some species, the proctiger is capable of being partially withdrawn within the genital segment. In other species, slight dorsal flexion is possible. These movements allow the protrusion of the aedeagus.

23. In those species where a penisfilum is present, it is normally coiled in a clockwise direction, when viewed dextrad. Anticlockwise coiling, to the same extent, occurs within the median penis lobe. As a result, when the penisfilum is extended, uncurling is unnecessary, and this organ and the adjacent portions of the ejaculatory duct merely undergo a compensating straightening of their parts.

24. Descriptions of the male genitalia of a number of Canadian species of Pentatomidae are given. Only those structures normally visible, and therefore of greatest practicable value are made use of.

25. In general, the present grouping of species in the various subfamilies of this order is in harmony with these findings. Of the species studied *Zicrona caerulea* and *Meadorus lateralis* exhibit the widest departures from the general features of their subfamilies.

26. Some suggestions on the general line of development of the genital structures are given. Evidences of primitiveness are suggested by: the direction of the opening of the genital-cup; size and degree of retraction of the eighth segment within the abdomen; degree of specialization of the proctiger, both as regards function and structure; the presence of genital plates; presence of bulb, penisfilum, median penis lobe, etc., on aedeagus, absence of superior and inferior ridge, etc.

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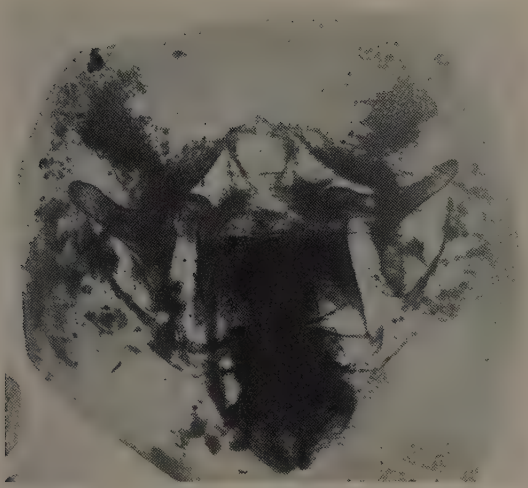

FIG. 156. *Photomicrograph of dorsal aspect of genital-cup of Euschistus euschistoides (treated specimen), with all organs in their normal positions.*

FIG. 157. *Photomicrograph of subdorsal aspect of the aedeagus of Euschistus tristigmus (penisfilum broken off).*

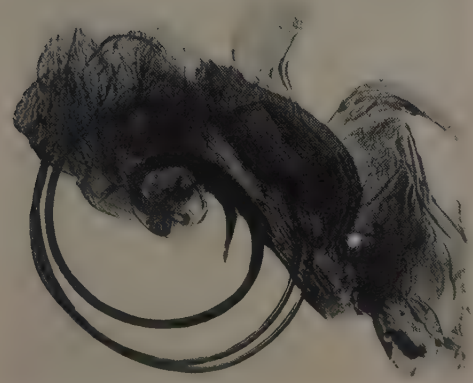

FIG. 158. *Photomicrograph of dextral aspect of the aedeagus of Euschistus euschistoides.*

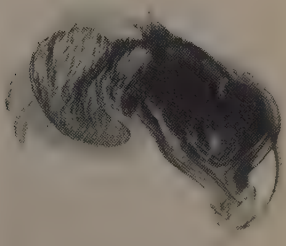

FIG. 159. *Photomicrograph of dextral aspect of aedeagus of Coenus delius Say.*

FIG. 160. *Photomicrograph of genital segment of Euschistus euschistoides with dorsum and proctiger removed to show comparative size of aedeagus. (Penisfilum showing horizontal instead of vertical coiling, owing to pressure of cover glass.)*

FIG. 161. *Photomicrograph of genital segment of Euschistus euschistoides with proctiger removed and aedeagus extended from the genital-cup. (Portion of "lip" removed.)*

FIG. 162. *Photomicrograph of chitinized internal structures of female of Euschistus euschistoides. Dorsal view. Terga of abdomen removed.*

STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES. XXXIV. THE CONSTITUTION OF LEVAN AND ITS RELATION TO INULIN[1]

By Harold Hibbert[2], R. Stuart Tipson[3], and F. Brauns[4]

Abstract

A description is given of the properties of levan, a polysaccharide obtained by the action of *Bacillus mesentericus*, and of its enzyme, on sucrose; in particular, its complete hydrolysis to fructose, and behavior when subjected to acetylation and methylation.

By hydrolysis of trimethyl levan, a crystalline trimethyl fructose was obtained (the first crystalline methylated γ-sugar to be synthesized). This yielded a trimethyl methylfructoside on condensation with methyl alcohol containing HCl. The properties of these compounds are described for the first time.

On methylation and subsequent hydrolysis of the glucide, a tetramethyl fructose was obtained having properties identical with those of the tetramethyl fructose obtained by hydrolysis of heptamethyl sucrose.

Oxidation of the new trimethyl fructose yielded a dibasic dimethyl lactol acid, and esterification of the latter, followed by methylation and treatment with dry ammonia, gave a crystalline diamide. These results show that the new crystalline trimethyl fructose is 1:3:4-trimethyl fructofuranose.

It follows from this that levan is a polymerized anhydro-fructofuranose with linkages at positions 2 and 6 of the fructose chain, thus differing from inulin which has been shown to be an anhydro-fructofuranose with linkages at positions 1 and 2.

The synthesis of levan thus represents a further example of the polymerization of an anhydro sugar taking place through the medium of the $\gamma\delta$ hydroxyl groups. It serves to emphasize the importance of these spatial positions with reference to the carbonyl group, a phenomenon to which attention has been drawn repeatedly by Hibbert.

Introduction

In the growing plant, the glucose units which later unite to form the starch and cellulose are, to a considerable extent, convertible into fructose, with the resulting formation of levulosans (5). Thus Colin and Belval (6), in the course of an investigation of the changes occurring in the stem of the wheat plant, found that after the plant has formed spikes, the stem contains an alcohol-insoluble substance, which on hydrolysis yields a large amount of fructose. Immature wheat grains contain 6% levulosan and an equal percentage of starch, but as maturity is approached, starch is formed in increasing quantities (50 to 60%) while the levulosan gradually drops to around 0.4%.

The presence of these polymerized anhydro-fructoses (levulosans) is not confined to any one group of plants, but their occurrence is widespread. The best known example of this class of substances is inulin, which has been shown by the able work of Haworth and coworkers (8, 19) to be a polymerized anhydro-fructofuranose, the linkages being at positions 1 and 2 of the fructose chain. Naturally, the existence of a number of possible polymers intermediate between fructose anhydride and inulin is to be expected, and many of them have, in

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[2] *Professor of Industrial and Cellulose Chemistry, McGill University.*
[3] *Research Fellow, Pulp and Paper Research Institute, Montreal.*
[4] *Research Assistant, Division of Industrial and Cellulose Chemistry, McGill University, Montreal.*

fact, been reported (45, 49, 50). Thus, the dimer, "sinistrin A" $(C_6H_{10}O_5)_2$ which occurs in *Scilla maritima* has been subjected to methylation (47) and the methylated product shown to give 3:4:6-trimethyl-fructofuranose on hydrolysis, a behavior analogous to that of trimethyl inulin. "Sinistrin A" has, indeed, since been obtained from inulin (46). "Sinistrin B" (47) $(C_6H_{10}O_5)_4$ is possibly identical with scorodose, which has been examined by Kihara (34).

Wood pulp contains about 2.5% levulosans, according to the work of Krause (37) and of Hägglund (15, 16) who found fructose among the sugars obtained from sulphite waste liquors as well as among the products of hydrolysis of spruce wood.

While bacteria possess the ability to synthesize levulosans from sucrose (2, 3, 4, 10, 12, 13, 14, 35, 36, 42, 43), no case appears to be on record in the literature indicating the bacterial production of a levulosan from glucose, such as appears to occur in the plant.

In particular, the polysaccharide known as levan* presents several features of peculiar interest to the student of polysaccharide chemistry since, as indidicated in a previous paper (17a), the bacteriological evidence appears to show that levan is only produced from those sugars containing the active or γ-ring form of fructose. It was therefore important to determine whether levan differs from inulin, (i) in the ring structure of the building unit, (ii) in the positions of union of contiguous building units, (iii) as regards the state of aggregation, or (iv) on account of traces of ash and other foreign substances.

Notwithstanding the fact that the literature of gum formation by bacteria is quite extensive, very few investigators have made a careful determination of the chemical and physical character of the gums obtained by them. Even when such has been made, no especial care seems to have been exercised, in the majority of cases, to ensure the efficient purification of the product, so that a rigid classification of their results seems to be impossible.

Our knowledge of the chemistry of levan is almost entirely due to the pioneer work of Greig-Smith (12, 13) although Kopeloff (35, 36) and also Owen (41, 42, 43), as indicated in a previous communication (17a), have since carried out a number of interesting investigations on the synthesis and properties of this substance. Greig-Smith arrived at the conclusion that levan (formed by the action of *Bacillus levaniformans* on sucrose) is formed from sucrose indirectly. He claimed that the organism first inverted sucrose and, from the nascent glucose and fructose thus formed, the organism produced the levan. He found that the organism could not produce levan from these sugars in their normal condition (pyranose).

Somewhat later Owen (41, 42, 43) conducted experiments on sucrose in-

**Objection to the name "gum levan".*

Levan (a) is only gummy when crude; (b) is not closely related to the group of substances usually designated as gums, and which contain sugar-acid residues in their molecule; (c) is much more closely related to polysaccharides such as xylan (formerly called "wood gum").

It is therefore proposed to dispense with the term "gum" as applied to levan, and to refer to it under the designation of "levan" thus making it analogous to araban, xylan, etc., to which class of substances it has now been shown to belong.

oculated with yeast and with gum-forming bacteria. He found that gum formation was much greater where bacteria were used alone than in the presence of yeast, thus apparently showing that the nascent fructose liberated by yeast is not in a suitable condition for polymerization. He claimed to have isolated the enzyme from *Bacillus vulgatus* and named it "levanase" as it produced levan from sucrose.

Kopeloff and coworkers (35, 36) found that mould spores contain a levan-forming enzyme, which exhibited maximum activity at a *p*H 7.0. They considered levan to be formed from nascent fructose and glucose.

Greig-Smith and Steel (14) gave an account of the physical properties of this type of levan. Their analysis agreed with the empirical formula $(C_6H_{10}O_5)_x$, the product having a m.p. of 200° C. A comparison of the properties of levan and inulin, taken from their paper, is given in Table I.

TABLE I

PROPERTIES OF LEVAN AND INULIN

	Levan	Inulin
$[\alpha]_D$	−40°	−40°
Oxidation (HNO_3)	Oxalic acid; no mucic acid	Oxalic acid; no mucic acid
Cold water	Very soluble	Sparingly soluble
Hot aqueous solution on cooling	Mucilaginous	Limpid
Prolonged heating with water at 100° C.	Unchanged	Hydrolysed to fructose
Hydrolysis with acid	Fructose	Fructose
Baryta water	Precipitate	Precipitate
Strontia water	Precipitate	—
Lime water	No precipitate	No precipitate
Lead subacetate	No precipitate	No precipitate
Ammoniacal lead acetate	Precipitate	Precipitate
Action of solution on PbO at room temperature	Does not dissolve PbO	Dissolves PbO
Ammoniacal $AgNO_3$ in dark	Not reduced	Reduced
Boiling Fehling's solution	Not reduced	Slowly reduced
Iodine	No action	No action
Cuprammonium hydroxide	Dissolves; does not precipitate on standing	Heavy precipitate on standing

In addition, levan is not precipitated by an acid solution of mercuric nitrate, nor by ferric chloride, copper sulphate nor tannic acid. It appears to be closely related to inulin and levulan but differs in important respects from both of these, as well as from derivatives of inulin variously described as pyro-inulin, metinulin, levulin, pseudo-inulin, inulenin, etc. (49).

Thus, levan differs from Lippmann's levulan (39), $[\alpha]_D = -221°$, obtained from beet-sugar vats: (a) in having a much smaller specific rotation; (b) in giving no blue precipitate with Fehling's solution; (c) in the fact that the concentrated solution does not gelatinize on cooling; (d) in not becoming insoluble in water after dehydration by alcohol; and (e) in not being precipitated by lead subacetate.

It was hoped that by the application of the well-recognized acetylation, methylation and hydrolysis technique, the exact relationship between levan and inulin might be determined definitely and that this, in turn, might lead to valuable information relating to the mechanism of polysaccharide formation in the plant.

Discussion of Results

Levan from one uniform source was employed, although other experiments, which will be communicated later, have been conducted on levan from a variety of sources. In the preliminary experiments levan prepared by the action of *Bacillus mesentericus* on sucrose was used, but when it was found (17a) that the enzyme preparation could be used for bringing about its formation, the latter method was employed exclusively since it gave a pure, homogeneous, ash-free product.

A specimen of anhydrous levan can be prepared by desiccation at 110° C. under diminished pressure, but the analysis of this anhydrous specimen is rendered difficult owing to its hygroscopic nature. Using the device of estimating the percentage of carbon and hydrogen in a moist (air-dried) sample, and then making allowance for the moisture content, values were obtained establishing the empirical formula $(C_6H_{10}O_5)_x$.

Complete hydrolysis by means of a dilute, aqueous oxalic acid solution gave 97% of the theoretical yield of syrupy fructose, thus proving that levan is composed entirely of fructose building units. (No definite yield of fructose from levan has been given by previous investigators, their statements being based on polarimetric data and qualitative tests.)

By precipitation of the levan from aqueous solution with alcohol*, it was obtained as a fine white powder, which was readily acetylated or methylated. On acetylation with acetic anhydride in the presence of pyridine, levan yielded a triacetate in the form of a white powder, having properties differing markedly from those of inulin triacetate.

TABLE II

COMPARISON OF THE TRIACETATES OF INULIN AND LEVAN

Triacetyl inulin (44)	Triacetyl levan
M.p. 102-3° C. (softens 95°)	Softens at 106°. Only completely liquefied at 196°
$[\alpha]_D = -42.55°$ in glacial acetic acid	$[\alpha]_D = +21.0°$ in chloroform
Found: C, 50.00* H, 5.60	Found: C, 49.92* H, 5.78
$(COCH_3)$, 44.53%	$(COCH_3)$, 44.33%

*Calcd. C, 49.98%; H, 5.6%; $COCH_3$, 44.79%.

De-acetylation of levan triacetate, by treatment with alcoholic potash, gave a quantitative yield of regenerated levan having properties identical with those of the original polysaccharide. In these respects the general behavior of levan seems to parallel that of inulin.

*Later work has shown that it is preferable to use methyl rather than ethyl alcohol.

A further comparison of the behavior of the two polysaccharides was made by methylation of the levan under conditions determined after numerous trials. It was found that the introduction of methoxyl groups to the extent of 40% was readily accomplished using an aqueous solution of levan in contact with dimethyl sulphate and potassium hydroxide. After completing the methylation by means of Purdie's reagents a trimethyl levan was isolated in a yield of 95% calculated on weight of levan used. Its properties are shown in Table III.

TABLE III

COMPARISON OF TRIMETHYL INULIN AND TRIMETHYL LEVAN

Trimethyl inulin (19)	Trimethyl levan
M.p. 138°-140° C.	M.p. 145°-147° C.
$[\alpha]_D = -50.2°$ in $CHCl_3$ (c. 1.61)	$[\alpha]_D = -89.0°$ in $CHCl_3$ (c. 1.11)**
Insoluble in water or ether	Insoluble in cold water or ether
Very soluble in benzene and acetone	Very soluble in benzene and acetone
Moderately soluble in ethyl alcohol	Moderately soluble in ethyl alcohol
Non-reducing to Fehling's solution	Non-reducing to Fehling's solution
Found: C, 53.1*	Found: C, 52.76*
H, 7.75	H, 7.84
OMe, 43.5%	OMe, 44.7%

*Calcd. C, 52.91%; H, 7.9%; OMe, 45.6%.

**Using NaOH for the preparation of the trimethyl levan a value of $-90.9°$ was obtained; with KOH the value was $-87.3°$. The combined mean value for various preparations was $-89.0°$.

The hydrolysis of trimethyl levan (I) yielded 92% of a crystalline trimethyl fructose (II) which had all the properties usually associated with a γ-sugar, including ease of combination with methyl alcohol in the presence of hydrogen chloride.

TABLE IV

COMPARISON OF 3:4:6-TRIMETHYL γ-FRUCTOSE AND 1:3:4-TRIMETHYL γ-FRUCTOSE

3:4:6-Trimethyl γ-fructose (19) (from trimethyl inulin)	1:3:4-Trimethyl γ-fructose (from trimethyl levan)
Colorless liquid	Colorless liquid; crystallizing to a solid, m.p. 73°
Decolorizes neutral $KMnO_4$ instantaneously	Decolorizes neutral $KMnO_4$ instantaneously
n_D^{14} 1.4675	n_D^{20} 1.4661 (liquid form)
$[\alpha]_D = +27.7°$ in $CHCl_3$ (c. 1.57)	$[\alpha]_D = +16.5°$ in $CHCl_3$ (c. 0.68)
Found: C, 48.45*	Found: C, 48.72*
H, 8.1	H, 8.4
OMe, 40.2%	OMe, 41.7%

*Calcd. C, 48.63%; H, 8.2%; OMe, 41.9%.

The product of the latter reaction, trimethyl methylfructoside, proved to be identical with that obtained by the hydrolysis of trimethyl levan with methyl alcohol containing hydrogen chloride.

The structure of this as trimethyl methyl-fructofuranoside (III) was established in the following manner: The substance was first submitted to a further complete methylation and the resulting tetramethyl methylfructoside (IV) then hydrolyzed. It yielded tetramethyl fructofuranose (V) identical in properties with that obtained from inulin (31, 32), from sucrose (1, 18) and from methyl fructofuranoside (40) and which has been shown by the researches of Avery, Haworth and Hirst (1) to contain a five-atom or furanose ring.

The fact, however, that the trimethyl γ-fructose from trimethyl inulin is a liquid while that from trimethyl levan is crystalline, pointed to a difference in the positions of methoxyl substituents, more especially since seeding of the former with a crystal of the latter did not bring about crystallization.

It was therefore decided to employ the well-established oxidation technique of Haworth and coworkers in order definitely to determine the positions of the methoxyl groups in the fructose chain.

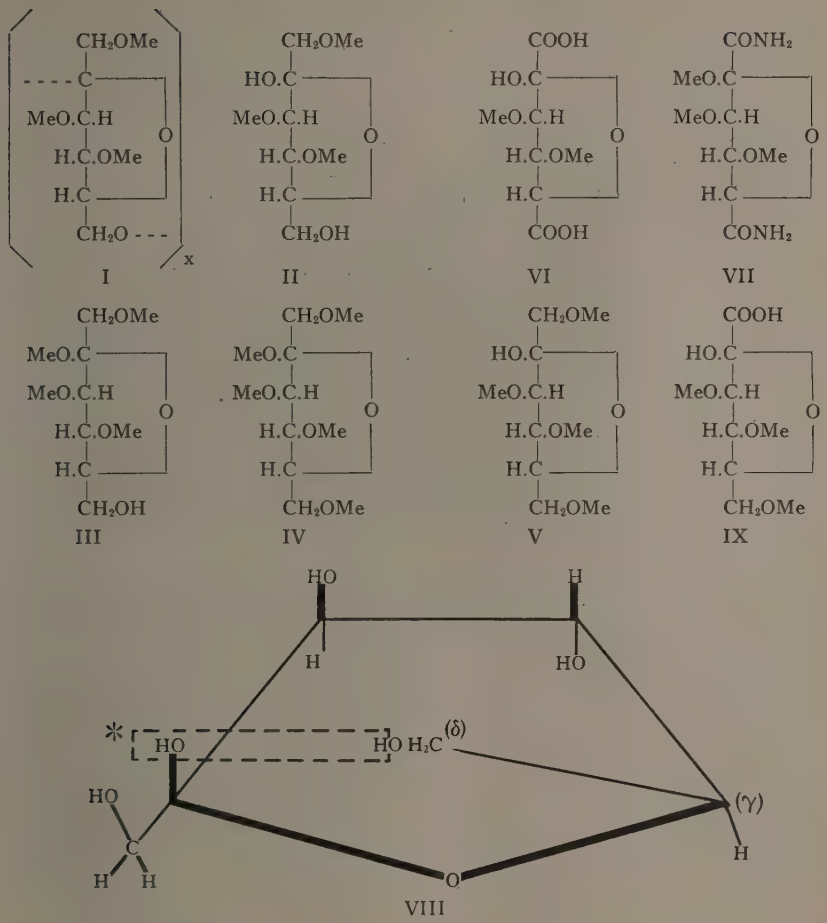
As shown by Haworth and Learner (19), oxidation of 3:4:6-trimethyl γ-fructose from trimethyl inulin yields a monobasic trimethyl lactol acid (IX). On the other hand, oxidation of the trimethyl γ-fructose from trimethyl levan gives a yield of 96.8% of a dibasic dimethyl lactol acid (VI), from which, on esterification with methyl alcohol followed by methylation with silver oxide and methyl iodide, a yield of 97.4% of the fully methylated, pure dimethyl ester was obtained. On treatment of this ester with a saturated solution of dry ammonia in methyl alcohol the corresponding diamide (VII) was obtained (yield 93%). The calcium salt of the dimethyl lactol acid was obtained as a crystalline product containing four molecules of water of crystallization.

It is thus apparent that the trimethyl fructose isolated is 1:3:4 trimethyl fructofuranose. This is confirmed by: (a) its inability to form an osazone (showing that position 1 is probably methylated), and the fact that (b) nucleation of authentic 3:4:6-trimethyl fructofuranose (from trimethyl inulin) with a crystal of the "levan" trimethyl γ-fructose does not produce crystallization.

From these experimental results the conclusion is drawn that levan is derived from a 2:6-anhydro-fructofuranose building unit (VIII) in which the chain formation takes place through the medium of carbon atoms 2 and 6. In other words, it represents a polymerization product of an anhydro-fructofuranose, the latter being formed by the loss of a molecule of water from the (CO) group and the (OH) groups in the γ and δ positions. The importance to be attached, in the study of polysaccharide formation, to the two hydroxyl groups in the γ and δ positions was first pointed out by Hibbert (22, 23) and the idea further extended by Hibbert and coworkers (23a, 24, 28).

The instability of the polysaccharide towards hydrolyzing agents, which is somewhat comparable to that of sucrose and inulin (27), would also tend to support the view that levan is a polymerized anhydro-fructofuranose.

At any rate, the readiness with which fructose combines with methyl alcohol to form the furanoside—one of the most rapid furanoside formations known (40)—and the fact that fructose (when combined) appears to occur in nature invariably in the furanose form, seem to indicate that the stereochemical

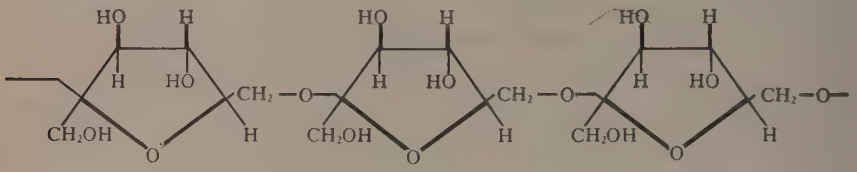


**Reducing group.*

grouping in fructose lends itself to the formation of furanose derivatives more readily than that of any other sugar occurring in the plant.

It is tentatively suggested that β-fructofuranose residues only are concerned in the levan complex, since the specific rotation of pure levan ($-42.6°$) is analogous to that of β-methyl fructopyranoside ($-172.1°$) but very different from that of α-methyl fructopyranoside ($+45°$). The hypothesis may be tested further when the indirect isolation of the crystalline α- and β- forms of methyl fructofuranoside has been accomplished. At present only the syrupy mixture of the α- and β- forms of methyl fructofuranoside has been prepared of $\left[\alpha\right]_D = +26.6°$ (40). The formation of levan from sucrose (the latter having recently been found by Hudson (29) to contain β-fructose residues) also suggests that levan may contain β-fructofuranose residues.

The contiguous units of levan may thus be depicted by the following:



(X)

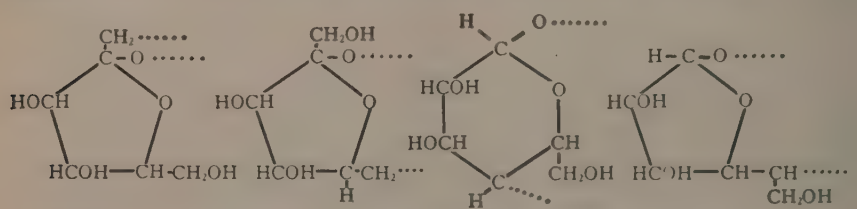
No attempt has been made in this research to arrive at a definite decision as to the size of the "levan aggregate", this work being reserved for a future communication dealing with molecular and aggregate size of polysaccharides in general. The recent communication on this subject by H. Pringsheim, to the Union Internationale de Chimie at Liège (September 1930) serves to indicate the necessity for a more thorough investigation of the field. In the opinion of the senior author the experimental evidence available would seem to point to some considerable difference in character in the nature of the products derived from a polymerized anhydro-aldose and a polymerized anhydro-ketose respectively.*

Experimental

In collaboration with Mr. H. L. A. Tarr (17a) levan was prepared from sucrose by the action of *Bacillus mesentericus* (48) and of its enzyme preparation. It was purified as indicated in a previous communication (17a).

Prepared in this manner, levan is a tasteless, ash-free, white powder which retains 7.73% of moisture when air-dry under average conditions of humidity (as determined by heating a sample at 110° C. in a microvacuum desiccator to constant weight). It softens at 182° C., and turns to caramel at about

**The interesting character of such polymerization products is evident from a consideration of the structure of levan, inulin, cellulose, starch, etc., from the point of view of the previous investigations carried out by Hibbert and coworkers on the role played by the γ:δ-hydroxyl groups in carbohydrates.*



I *Inulin* II *Levan* III *Cellulose; starch; glycogen* IV *Dextran (?)*

It will prove of considerable interest should "dextran", as surmised, be found to possess the structure of a polymerized glucofuranose. Preliminary investigations point to the existence of a considerable number of dextrans some of which apparently represent polymerized products containing glucose anhydride units combined with an acid.

Such changes as those indicated in this, and the previous communication, make it appear not improbable that they may later prove to have a bearing on the subject of cancer metabolism, since they represent irreversible processes.

Cell proliferation may stand in intimate relation to abnormal, irreversible conversion of the free radicle or a less stable cyclic form of one or more carbohydrates or related derivatives, and its further metabolism along abnormal lines. (H. H.)

200° C. Levan is insoluble in most organic liquids but dissolves readily in hot chloral, glycerol and ethylene glycol, which are characterized by being good solvents for polymerized anhydro-sugars.

It dissolves readily in hot water to give a neutral solution, a dilute solution being faintly opalescent. It shows no coloration with iodine and is without action on Fehling's solution either at room temperature during a period of 48 hr. or even on prolonged boiling. It is readily hydrolyzed by dilute acids to give fructose, characterized by the usual color tests. The pure aqueous solution of the moisture-free material has a rotation of $[\alpha]_D^{22} = -45.3°$ in water (c = 2.2).

Levan retains some 3.2% moisture even when dried at 78° C. under diminished pressure over phosphoric anhydride for 12 hr., but this is lost on heating for several hours at 110° C./15 mm. in a microvacuum desiccator (*e.g.*, 8.079 mg. gave 7.821 mg. % $H_2O = 3.19\%$).

Analysis: 4.491 mg. of the sample dried at 78° C. gave 2.607 mg. H_2O and 7.056 mg. CO_2. These figures, when corrected for moisture, correspond to C = 44.26; H = 6.3%; calcd. for $(C_6H_{10}O_5)_x$; C = 44.42; H = 6.2%.

The Action of So-called "Levanase" on Sucrose

The action of levanase on sucrose was studied by estimating the free glucose formed, using the method of Willstätter and Schudel (52). To an accurately prepared sterile solution of sucrose in water (about 10%) was added 1 gm. of enzyme preparation per litre of solution, the latter covered with toluene and incubated at 37.5° C., shaking once every 24 hr. The amount of glucose in 10-cc. test samples was then estimated from day to day, each titration being

TABLE V

RATE OF CONVERSION OF SUCROSE INTO LEVAN BY ENZYME ACTION

Time in days	*N*/10 iodine equivalent to 10-cc. sample, in cc.	% sucrose transformed
Solution I (101.21 gm. sucrose per litre)		
1	3.4	5.8
2	4.7	8.0
4	6.9	11.6
5	8.8	14.9
6	9.2	15.5
7	9.7	16.9
8	10.3	17.5
Solution II (98.75 gm. sucrose per litre)		
7	10.4	18.0
14	12.9	22.3
15	13.2	22.9
18	13.1	22.7
19	13.1	22.7
21	13.4	23.2
22	13.7	23.7
24	13.7	23.7

run in triplicate. It was observed that the opalescence increased proportionately to the increase in free glucose content so that the reaction could not be followed by observation of changes in specific rotation. The results are given in Table V.

The figures in Table V illustrate the rate of formation of glucose and this in turn presumably provides an indication of the rate of levan formation. Assuming that no free fructose is actually liberated, it provides, indeed, an absolute measure of levan formation.

*The Hydrolysis of Levan**

An approximately 10% aqueous solution of levan was hydrolyzed with 0.5% oxalic acid by heating at 100° C. for one hour, using the method of Düll (9). The hot solution was then made neutral by addition of finely powdered calcium carbonate, and filtered. The filtrate was treated with a little decolorizing charcoal, filtered and evaporated under diminished pressure, below 40° C., to a thick syrup, the usual precautions being observed to prevent any rise in temperature during the process in order to avoid decomposition of the fructose. The syrup was dissolved in boiling absolute ethyl alcohol under reflux condenser, the solution again evaporated to dryness and the resulting anhydrous sugar weighed. Yield of fructose syrup = 10.20 gm. from 9.46 gm. dry levan (97.1% of theory).

The syrup was dissolved in the minimum amount of boiling absolute alcohol under reflux condenser and allowed to cool slowly in a vacuum desiccator over calcium chloride. Colorless crystals (m.p. 99° C. uncorr.) separated in good yield. $\left[\alpha\right]_D^{21} = -102.4°$ (four minutes after dissolving in water) decreasing to $-90.75°$ (c = 1.18). The latter figure is in fair agreement with the value $-88.7°$ calculated from the formula of Jungfleisch and Grimbert (33) for fructose. Analysis: H = 7.0; C = 40.03%; calcd. for $C_6H_{12}O_6$; H = 6.7; C = 39.98%.

Preparation of Levan Triacetate

Levan (18 gm.) was heated under reflux for two minutes (or until a vigorous action commenced) with 100 cc. pyridine and 70 cc. acetic anhydride. When the ebullition had subsided (leaving about 0.5 gm. levan undissolved) the mixture was poured slowly into 1,600 cc. of ice water, with vigorous mechanical stirring. After standing in the refrigerator for three hours the greater part of the supernatant liquor was decanted from the white, powdery precipitate. The latter was then drained and washed twice with ice water using suction. It was dried in a vacuum desiccator over phosphorus pentoxide. Yield of anhydrous material, 17.4 gm.; it is a tasteless, white powder, m.p. crude, 193° C.

It was purified as follows: 9.5 gm. of the finely powdered acetate was refluxed twice with a fresh volume of 100 cc. dry ether for 20 min. to remove any traces of ether-soluble acetates. (The extract on evaporation always yielded a negligible amount of a pale yellow syrup.) The extracted levan acetate was then dissolved by refluxing with 50 cc. chloroform and the hot solution filtered

**For a complete polarimetric record of the hydrolysis of levan see (27).*

through glass wool, when necessary. The filtrate was poured into five volumes of absolute alcohol, with vigorous mechanical stirring. The white precipitate was allowed to settle, filtered through a sintered-glass Gooch crucible and freed from alcohol by washing on the filter with dry ether. It was then dried in a vacuum desiccator over phosphoric anhydride; it softens at 106° C., and is completely liquefied at 196° C.

Pure levan acetate is soluble in cold chloroform, glacial acetic acid, pyridine, and chloral. It is sparingly soluble in hot acetone, methyl alcohol, ethyl alcohol, glycerol and benzene; readily soluble in hot camphor, and insoluble in cold and hot ether, ethyl acetate, petroleum ether, tetrachloroethane, water, ethylene glycol, ethylene glycol mono ethyl ether. $\left[\alpha\right]_D^{22} = +21.0°$ in chloroform (c = 2.58).

Analysis: Found: C = 49.92; H = 5.78; $COCH_3$ = 44.33%; calcd. for $(C_{12}H_{16}O_8)_x$: C = 49.98; H = 5.6; $COCH_3$ = 44.79%.

Regeneration of Levan from the Acetate

Four grams of levan triacetate was shaken for 30 min. with 100 cc. *N*/2 alcoholic potassium hydroxide at room temperature. The excess alkali was then neutralized with glacial acetic acid, the regenerated levan filtered off, and rubbed with alcohol containing a little acetic acid until the whole of the alkali had been removed. The levan was now dissolved in 20 cc. distilled water and run into 400 cc. absolute ethyl alcohol with vigorous mechanical stirring. The resulting fine, white, powdery precipitate was triturated with absolute alcohol and dried at 78° C. under reduced pressure. So obtained it was a tasteless, white powder, non-reducing towards boiling Fehling's solution, and possessing properties identical with those of the original levan.

The Methylation of Levan

Preliminary experiments in which levan was methylated with dimethyl sulphate in the presence of sodium hydroxide having indicated that under these conditions the methylation was slow and tedious, recourse was had to the use of potassium hydroxide in place of sodium hydroxide. This proved eminently satisfactory (17).

Thirty grams of levan was dissolved in 60 cc. distilled water, and the solution introduced into a three-necked flask provided with a mechanical stirrer and two dropping funnels. It was then methylated by means of 130 cc. dimethyl sulphate and 370 cc. of 30% aqueous potassium hydroxide solution, each added in 10 equal portions as follows: the first two additions, made dropwise, were carried out at room temperature over a period of one hour for each, with vigorous mechanical stirring, the mixture always being kept faintly alkaline. The temperature was then raised to 40° C. and the time for the next additions was diminished to 30 min. each. The temperature was gradually raised and at the sixth addition (temp. = 60° C.) an insoluble, white, syrupy product settled out on the surface, so the stirrer was readjusted to carry this down into the methylating liquor. Subsequent additions were made every 15 min. and the temperature was raised to 70° C. Thereafter the temperature was maintained at 100° C. for one hour, with vigorous stirring and during this period

the insoluble substance settled to the bottom as a fine powder (A), quite white in color.

The hot supernatant liquor was carefully decanted, cooled and extracted three times by shaking with chloroform. The united chloroform extracts were dried with anhydrous magnesium sulphate, filtered, and the filtrate evaporated to a syrup under diminished pressure at 30° C. The product so obtained was added to the solid (A) in the methylating flask and the whole re-methylated with the same amounts of dimethyl sulphate and potassium hydroxide as described, using efficient mechanical stirring to keep the solid in constant motion through the liquid. The additions of the methylating agents in one-tenth portions were made every 15 min., the temperature, initially at 50° C., being gradually raised to a final value of 70° C. The mixture was then heated in the boiling water bath for one hour, with vigorous mechanical stirring.

The supernatant liquor was again decanted from the fine white powdery precipitate (B), and extracted with chloroform as before. To free it from occluded potassium sulphate, the solid (B) was filtered, pressed dry and was then extracted three times under reflux with boiling chloroform.

The united chloroform extracts were dried over anhydrous magnesium sulphate, filtered and the filtrate evaporated to a thick syrup under diminished pressure at 30° C. This was obtained as a white powder by refluxing with dry ether for 30 min. The powder was filtered, and dried in a vacuum desiccator at 78° C. over phosphorus pentoxide under diminished pressure. Yield = 36.8 gm. Analysis: Found: OMe = 40.7%; calcd. for $(C_9H_{16}O_5)_x$; OMe = 45.6%.

This substance was subjected to one further methylation by means of Purdie's reagents. In order to prevent undesirable side reactions (as apparently obtained by Irvine in the methylation of inulin (31, 32)) a slight modification of the method, as used by Hibbert and coworkers (25, 26), was introduced, whereby the silver oxide was kept in suspension by vigorous mechanical stirring. It was added in portions from time to time, instead of introducing the full amount in one operation, it having been observed that this diminishes the tendency for the active surface of the silver oxide to become vitiated before it has taken part in the methylating reaction. In all cases examined it was found that the number of treatments with Purdie's reagents could then be considerably reduced and the yield increased.

The reaction was carried out in a three-necked flask through the middle neck of which was inserted a motor-driven stirrer having a mercury seal; the second opening was provided with a water condenser closed by a tube filled with calcium chloride, while the third neck, closed by a cork, served for the introduction of the silver oxide.

The product to be methylated was dissolved in methyl iodide and introduced into the flask, which was then placed in a thermostat at 47° C. The freshly prepared silver oxide (dry and finely powdered) was added in portions of one-tenth of the total quantity, a new addition being made every 30 min.

After the addition of all of the silver oxide the reaction was allowed to proceed

during a further one hour, the stirrer being so adjusted as to prevent the formation of a layer of silver oxide at the bottom of the flask. Under these conditions very efficient methylation took place.

Chloroform was now added and the mixture stirred for 15 min., after which the mixture was filtered and the silver salts extracted four times with boiling chloroform under reflux. The united chloroform extracts were dried with anhydrous magnesium sulphate, filtered and the filtrate evaporated under diminished pressure at 30° C., yielding a pale yellow, glass-like solid. This was refluxed with dry ether for 30 min. to dissolve the trace of yellow coloring matter present and at the same time to precipitate the trimethyl levan as a white powder. The ether was decanted, the powder washed several times with dry ether by decantation, and dried in a vacuum desiccator over phosphorus pentoxide. Yield 36 gm., (95% of theory), m.p. 145°-147° C., $[\alpha]_D^{23} = -87.3°$ in tetrachlorethylene (c = 2.75). A second preparation had $[\alpha]_D^{21} = -90.9°$ (c = 2.9).

Thus prepared, trimethyl levan is a tasteless white powder which is non-reducing to boiling Fehling's solution. It is soluble in chloroform, tetrachloroethane, benzene, glacial acetic acid, carbon tetrachloride, absolute ethyl alcohol, acetone, methyl iodide; soluble in boiling water and boiling methyl alcohol to give a faintly opalescent solution; insoluble in ether, petroleum ether and aqueous alkali. For analysis, trimethyl levan was dried in a vacuum over phosphorus pentoxide at 78° C. for 24 hr. Analysis: Wt. 0.0193 gm. gave 0.0699 gm. AgI; OMe = 44.7%. Wt. 0.0235 gm. gave 0.0169 gm. H_2O and 0.0456 gm. CO_2; H = 8.05; C = 52.9%; calcd. for $(C_9H_{16}O_5)_x$; OMe = 45.6, H = 7.9, C = 52.91%.

Hydrolysis of Trimethyl Levan; Preparation of 1:3:4-Trimethyl Fructofuranose (II)

Ten grams of trimethyl levan was dissolved in 500 cc. of 70% aqueous ethyl alcohol by boiling under reflux, on a water bath at 95° C. The solution was cooled and the rotation observed.

Anhydrous oxalic acid (5.0 gm.) was now added, and the solution again refluxed, the rotation being observed at appropriate time intervals by cooling to room temperature and removing a sample, which was returned to the main solution after the observation.

TABLE VI

COURSE OF THE HYDROLYSIS OF TRIMETHYL LEVAN

Time of refluxing in hr.	$[\alpha]_D^{21}$	Time of refluxing in hr.	$[\alpha]_D^{21}$
0.0	−43.28	5.5	−24.24
0.5	−41.55	7.5	−19.04
1.5	−38.08	13.0	−17.31
2.5	−34.52	14.0	−17.31

After some 13 hr. the rotation remained constant and the hot solution was then made neutral to Congo paper by addition of freshly precipitated calcium

carbonate. The calcium salts were allowed to settle, filtered, and the filtrate evaporated under diminished pressure at 30° C. (The calcium salts on the filter were extracted three times under reflux with absolute ethyl alcohol and the extract also evaporated and the syrup added to the main portion.)

The resulting product was extracted three times under reflux with boiling chloroform and the extract, after drying over anhydrous magnesium sulphate, evaporated under diminished pressure, yielding a very pale yellow syrup (wt. = 10.8 gm.). This proved to be a mixture of 1:3:4-trimethyl fructofuranose and its ethylglucide; it was therefore completely hydrolyzed by heating under reflux with a mixture of 140 cc. *N*/10 HCl and 60 cc. distilled water for two hours at 80° C. The solution was cooled, and rendered neutral to Congo paper by addition of finely powdered silver carbonate. The precipitated silver chloride was filtered, pressed dry and extracted three times with boiling chloroform under reflux. Evaporation of this extract gave a syrup which was added to the main product. The aqueous filtrate was evaporated under diminished pressure, giving a pale yellow syrup which was extracted with dry ether to free it from traces of mineral salts. This ethereal solution was evaporated under diminished pressure at 30° C. yielding a pale yellow syrup (10 gm.) which was subjected to distillation under high vacuum. At a bath temperature of 125-135° C. and a pressure of 0.05 mm. the product distilled at 115-116° C. as a colorless, fairly mobile, homogeneous liquid, $n_D^{20} = 1.4661$; wt. 9.5 gm.

It reduced Fehling's solution strongly, and decolorized cold neutral potassium permanganate solution instantaneously. This oil crystallized after standing in the refrigerator overnight and scratching with a glass rod.* Star-like clusters of long needles separated, whereupon the entire material solidified. It was purified by recrystallizing twice from carbon tetrachloride; m.p. 73° C. (uncorr.), and was found to possess an extremely bitter taste.

This pure crystalline trimethyl fructose reacts extremely slowly with a cold, neutral, dilute solution of potassium permanganate. If, however, a little of the crystalline material be gently warmed in a bath at 70° C. until molten, then allowed to cool, it was found that the resulting syrup† then decolorized a cold, neutral, dilute, aqueous potassium permanganate solution with extreme rapidity.** The syrupy and the crystalline sugar show equal reducing power towards Fehling's solution. It is of interest that Haworth and Mitchell (20), using tetramethyl γ-fructose, found that in alkaline solutions of potassium permanganate decolorization proceeds smoothly and readily. Crystalline 1:3:4-trimethyl fructofuranose decolorizes cold alkaline permanganate solution very rapidly and much faster than in the absence of alkali. $[\alpha]_D^{22} = +16.5°$

*In all subsequent preparations the crude sugar crystallized so that purification by distillation under high vacuum was unnecessary.

†The crystalline α-and β-ethyl glucofuranosides have no effect on potassium permanganate (21), whereas the syrupy form of γ-methylglucoside speedily decolorizes it (11, 30).

**This behavior is to be more closely investigated. It is intended to compare the "activity" of the syrupy and crystalline forms of 1:3:4-trimethyl fructofuranose with that of 3:4:6-trimethyl fructofuranose prepared from inulin, using the method of Whitnah and Milbery (51) which is a modification of that of Kuhn and Wagner-Jauregg (38).

in chloroform (c=6.45). In carbon tetrachloride $[\alpha]_D^{22} = +21.9°$ (c=4.35). Molecular weight in benzene=213; 224 (calcd. 222). Analysis: Wt. 0.02495 gm. gave 0.0787 gm. AgI; OMe=41.7%. Wt. 0.0202 gm. gave 0.0150 gm. H_2O and 0.0361 gm. CO_2. H=8.3%; C=48.7%; calcd. for $C_9H_{18}O_6$; OMe=41.9; H=8.2; C=48.63%.

TABLE VII

MUTAROTATION IN WATER OF CRYSTALLINE 1:3:4-TRIMETHYL FRUCTOFURANOSE

Time (in min.)	$[\alpha]_D^{21}$ (c=8.1)	Time (in min.)	$[\alpha]_D^{21}$ (c=8.1)
4	−23.8	17	−40.7
5	−26.5	20	−48.3
6	−29.2	30	−51.4
		60	−51.85

Attempts to prepare an osazone were unsuccessful, although parallel experiments performed on an authentic sample of 3:4:6-trimethyl fructofuranose (obtained from natural inulin), under exactly the same conditions, readily gave 3:4:6-trimethyl fructofuranosazone in good yield in every case*. This had the properties described by Haworth and Learner (19).

Preparation of 1:3:4-Trimethyl Methylfructoside (III)

1:3:4-trimethyl methylfructofuranoside was obtained both from 1:3:4-trimethyl fructofuranose and from trimethyl levan.

(a) Preparation from 1:3:4-trimethyl fructofuranose (II). 1.8 gm. of 1:3:4-trimethyl γ-fructose was dissolved in 90 cc. dry methyl alcohol, 2.1 cc. of dry methyl alcohol containing 12 gm. hydrogen chloride per 100 cc. added, and the solution rapidly diluted to 100 cc. with dry methyl alcohol. The solution was maintained at room temperature, the rotation being observed immediately and after appropriate time intervals.

TABLE VIII

FORMATION OF 1:3:4-TRIMETHYL METHYLFRUCTOFURANOSIDE (III)

Time	$[\alpha]_D^{22}$	Time	$[\alpha]_D^{22}$
2 min.	−11.55	4.5 hr.	+28.87
43 min.	− 1.93	7.5 hr.	36.56
75 min.	+ 5.77	8.25 hr.	38.49
93 min.	9.63	18.75 hr.	46.19
2 hr.	13.48	19.25 hr.	+46.19

**In order to obtain authentic 3:4:6-trimethyl fructofuranose and its derivatives for purposes of comparison, the whole of the work on inulin by Haworth and Learner (19) has been repeated. All the data given by these authors have been verified but it has been found that, although a trimethyl inulin having the properties ascribed by them to this substance can be obtained if inulin, carefully purified by the method of Drew and Haworth (8), be methylated under the conditions they prescribe, it is nevertheless possible to prepare a trimethyl inulin, which is readily and completely soluble in ether, if the inulin be methylated exactly as described above for levan. That this procedure involves no change in ring structure but, presumably, is merely due to a lower state of aggregation is revealed by the fact that this ether-soluble trimethyl inulin gives a quantitative yield of 3:4:6-trimethyl fructofuranose on hydrolysis (32). Possibly this difference in property may be due to a difference in the prior treatment of the inulin during its purification.*

When the rotation was constant the reaction was arrested by the addition of dry, finely powdered silver carbonate and shaking until the solution was neutral to Congo paper. The mixture was filtered and the filtrate evaporated under diminished pressure at 30° C., yielding a pale yellow syrup. (The silver chloride was extracted three times with chloroform under reflux, the extract being added to the main product.) Distillation under high vacuum gave a colorless, fairly mobile syrup, boiling at a bath temperature of 108°-109° C. /0.024 mm.; yield 1.6 gm.; $n_D^{22} = 1.4522$; $[\alpha]_D^{23} = +41.59°$ in $CHCl_3$ (c = 2.00), $[\alpha]_D^{23} = +57.35°$ in water (c = 1.09). It was non-reducing to boiling Fehling's solution but rapidly decolorized a cold, neutral solution of potassium permanganate.

(b) Preparation from trimethyl levan. Trimethyl levan (9.0 gm.) was dissolved in 450 cc. anhydrous methyl alcohol by boiling under reflux. To the cooled solution was added 30 cc. of a solution containing 12 gm. HCl per 100 cc. methyl alcohol, the solution diluted to 500 cc. with anhydrous methyl alcohol and the rotation taken immediately. This solution was then boiled under reflux for one hour, after which the rotation remained constant. The acid was neutralized by addition of silver carbonate and the filtrate evaporated to a syrup which was freed from traces of mineral impurity by solution in ether and filtration. Removal of the ether and distillation of the resulting syrup at a bath temperature of 108°-115° C. 0.018 mm. yielded a colorless, fairly mobile syrup (wt. 8.7 gm.), $n_D^{22} = 1.4520$. This was neutral to boiling Fehling's solution, but rapidly decolorized a cold neutral solution of potassium permanganate. Analysis: Wt. 3.420 mg. gave 13.47 mg. AgI; OMe = 52.04%. Wt. 4.850 mg. gave 3.675 mg. H_2O and 8.991 mg. CO_2; C = 50.56; H = 8.5%; calcd. for $C_{10}H_{20}O_6$; C = 50.81; H = 8.54; OMe = 52.54%.

Preparation of Tetramethyl γ-Methylfructoside (IV)

4.7 gm. of 1:3:4-trimethyl methylfructofuranoside was methylated by Purdie's reagents by the method outlined. Distillation of the methylation product yielded a colorless, very mobile syrup at a bath temperature of 87° C./0.013 mm.

The main fraction, wt. = 4.1 gm. ($n_D^{23} = 1.4447$), showed no reducing action towards boiling Fehling's solution, but decolorized cold, neutral potassium permanganate solution very rapidly; $[\alpha]_D^{23} = +29.53°$ in absolute ethyl alcohol (c = 0.82).

Analysis: Wt. 4.678 mg. gave 3.737 mg. H_2O and 8.952 mg. CO_2; H = 8.9; C = 52.19%; calcd. for $C_{11}H_{22}O_6$; H = 8.9; C = 52.76%.

Preparation of Tetramethyl γ-Fructose (V)

Tetramethyl γ-methyl fructoside (1.6 gm.) was dissolved in 100 cc. of 0.25% hydrochloric acid and heated at 80° C. for three hours, after which time the rotation had become constant. The acid was neutralized by addition of silver carbonate, and the filtrate evaporated to a syrup under diminished pressure. This product was extracted with dry ether, the extract filtered, evaporated to

a syrup under diminished pressure and distilled, giving a colorless, very mobile liquid ($n_D^{22} = 1.4531$) at a bath temperature of 101° C./0.030 mm.

The tetramethyl γ-fructose thus isolated showed a strong reducing action towards Fehling's solution and decolorized cold neutral potassium permanganate solution instantly. At equilibrium, $[\alpha]_D^{22} = +30.61°$ in water (c = 2.33). Analysis: Wt. 4.888 mg. gave 3.846 mg. H_2O and 9.157 mg. CO_2; H = 8.8; C = 51.09%; calcd. for $C_{10}H_{20}O_6$; H = 8.54; C = 50.81%.

Oxidation of Trimethyl Fructose (II) with Nitric Acid

Three lots of 5 gm. each of 1:3:4-trimethyl γ-fructose were each treated with 30 cc. of concentrated nitric acid (d = 1.42) in a water bath at 20° C. The temperature was then gradually increased. At 65° C. gas evolution became vigorous. The solution was kept at this temperature until the reaction had subsided. The temperature was then slowly raised to 95° C. during a period of 1½ hr. from the commencement of the reaction.

The combined solutions were cooled, diluted with five times the volume of cold distilled water and the solution evaporated under diminished pressure (12 mm.) with continuous addition of water. This procedure was repeated many times until the final distillate was free from nitric acid. This occupied six days' continuous distillation. The dimethyl lactol acid so obtained was left as a thick yellowish syrup (wt. 17.5 gm.), the purification of which proved very difficult. In order to identify it, the calcium salt was prepared by dissolving 0.5 gm. of the syrupy residue in 10 cc. distilled water and neutralizing the boiling solution with pure calcium carbonate. The mixture was filtered and the filtrate allowed to evaporate spontaneously in a vacuum desiccator over calcium chloride. The crystalline calcium salt of the lactol acid separated as a hard crust of polyhedral crystals. These were recrystallized from hot water (yield 0.6 gm.). The salt crystallized with four molecules of water of crystallization. Analysis: 0.2330 gm. lost 0.0478 gm. water on drying in vacuum at 100° C. over P_2O_5. Found: H_2O, 20.5% (calcd. for $(C_8H_{10}O_8)Ca.4H_2O$, 20.8%). 0.0696 gm. of the anhydrous calcium salt gave on ignition 0.0142 gm. CaO; 0.0285 gm. gave 0.0464 gm. AgI. Found: 14.58% Ca and 21.5% OMe; (calc. for $(C_8H_{10}O_8)Ca$: Ca, 14.6%; OMe, 22.5%).

Conversion of the Dimethyl Lactol Acid into the Methyl Ester

The remaining 17 gm. of the syrupy dimethyl lactol acid was first freed from water by solution in absolute methyl alcohol and distillation of the solution. The syrupy residue was then dissolved in 250 cc. absolute methyl alcohol containing 3% HCl, and refluxed for 8 hr. After cooling the solution in ice-water, the HCl was removed by shaking with silver carbonate. The filtrate was evaporated under diminished pressure (12 mm.) and the small amount of silver salts left in the residue then removed by dissolving in dry ether and filtering. The filtrate on evaporation left a yellowish mobile liquid which on distillation under high vacuum (0.05 mm.) distilled at 110-120° C.; yield 18.5 gm.

The product appeared to be a mixture of the di- and trimethyl lactol acid

dimethyl esters. This was confirmed by the methoxyl determination, which showed 52.1% OMe, while the theoretical values for the di- and the trimethyl lactol acid dimethyl esters are 47.0 and 55.77% respectively. It was therefore remethylated using silver oxide and methyl iodide.

Preparation of the Trimethyl Lactol Acid Dimethyl Ester

Sixteen grams of the mixture of the di- and trimethyl lactol acid dimethyl esters were dissolved in 100 gm. MeI and 68 gm. of silver oxide added in ten portions during the course of 5 hr., the temperature being maintained at 46-47° C. After the last addition the reaction was allowed to continue for 1 hr. at the same temperature. The mixture was cooled, dry ether added and the mixture was again cooled and filtered. The silver oxide was washed with dry ether, the united ether extracts dried with anhydrous magnesium sulphate, filtered and evaporated. The yellowish residue was distilled at 0.04 mm., b.p. 112° C.; $n_D^{22} = 1.4529$; yield 16.5 gm. (98%). Analysis: 0.0212 gm. gave 0.01255 gm. H_2O and 0.0367 gm. CO_2; 0.0205 gm. gave 0.0854 gm. AgI. Found: H = 6.62; C = 47.21; OMe = 55.04; (calcd. for $C_{11}H_{18}O_8$: H = 6.5; C = 47.4; OMe = 55.8%); $[\alpha]_D^{22} = -19.4°$ in absolute alcohol (c = 10.8).

Preparation of the Diamide of the Trimethyl Lactol Acid

The ester (6.5 gm.) was dissolved in 50 cc. absolute methyl alcohol, the solution cooled in an ice-salt mixture and saturated with dry ammonia. After standing in the refrigerator for 3 days at 0° C. the solution solidified to a mass of star-shaped silky needles. They were filtered off and recrystallized by dissolving in boiling ethyl alcohol, cooling, and adding ether. Yield, including pure diamide recovered from the mother liquors, 93%; m.p. 192° C. Analysis: 0.1156 gm. was distilled with KOH. The ammonia evolved corresponded to 9.3 cc. $N/10$ H_2SO_4, equivalent to 13.73% NH_3 or 11.3% nitrogen. Theory for $C_9H_{16}O_6N_2$, N = 11.3%. 0.0204 gm. gave 0.0571 gm. AgI, OMe = 37.0% (calcd. for $C_9H_{16}O_6N_2$, OMe = 37.5%). $[\alpha]_D^{22} = -73.5°$ (in water, c = 8.1).

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STUDIES ON LIGNIN AND RELATED COMPOUNDS. PART VII. A KINETIC STUDY OF THE ACTION OF HYPOCHLOROUS ACID ON SPRUCE LIGNIN AND ITS BEARING ON THE CONSTITUTION OF THE SPRUCE LIGNIN MOLECULE[1]

By Harold Hibbert[2] and K. Austin Taylor[3]

Abstract

The action of hypochlorous acid on glycol-lignin in finely divided aqueous suspension, and in alkaline solution, is autocatalytic, the autocatalysis being due to formation of hydrochloric acid, which catalyzes the reaction strongly. Nitric acid apparently acts only by increasing the concentration of undissociated hydrochloric acid. The effect of the hydrochloric acid may be a direct catalytic effect of undissociated hydrochloric acid, or may be due to formation of chlorine.

A comparison of the amounts of hypochlorous acid absorbed, and hydrochloric acid formed, by methylated and unmethylated lignin indicates that if ketomethylene groups are present in lignin they do not enolize sufficiently to play any appreciable part in the rapid reaction between hypochlorous acid and lignin.

The rapid absorption of hypochlorous acid by lignin, dissolved in alkali, indicates that there is probably about 15% of a phenol nucleus in lignin. This value is supported by the rapid absorption of bromine from bromine water at very low concentrations, by lignin dissolved in alkali, and is based on the assumption of only one atom of halogen entering the phenol nucleus, which is true in the case of vanillin. If two or three halogen atoms enter the nucleus, this value of 15% has to be divided by two or three. It represents, in any case, a maximum value.

The phenol nucleus accounts for only one third, or less, of the total rapid hypochlorous acid absorption. The remainder is probably due to addition of hypochlorous acid to an ethylene linkage, chlorination of a phenol ether, or a combination of both reactions.

Introduction

Up to the present a knowledge of the constitution of spruce lignin has resolved itself into an identification of certain groupings and, due to the variety of methods employed in its isolation from wood, the data is for the most part indirect and problematical.

In the past, the method of attack has been purely organic, and in view of the inherent difficulties involved in such an investigation, a kinetic study of the problem was undertaken in an attempt to prove, if possible, the existence of certain groups presumed present in lignin, particularly an ethylene linkage, a phenolic nucleus and a tautomeric ketomethylene group.

The fact that practically all of the woody structure called "lignin" reacts readily with chlorine water led to the selection of this reaction as being well suited to a kinetic study of the problem.

Before, however, a description and discussion of the kinetic study is undertaken it seems advisable to review briefly the available evidence for the existence of the above mentioned groupings in the lignin complex.

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Contribution from the Industrial and Cellulose Chemistry Laboratories, McGill University, Montreal, Canada. From a thesis presented by K. Austin Taylor, in May 1929, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] *Professor of Industrial and Cellulose Chemistry, McGill University.*

[3] *Postgraduate student, McGill University, and holder of the Abitibi Power and Paper Company Research Fellowship.*

Available Evidence for the Existence of Certain Groups in the Lignin Complex

Ethylene Linkage

Most of the constitutional formulae proposed for lignin contain one or more ethylene linkages. The evidence for the presence of this grouping, however, is not very conclusive, and rests mainly on the formation of sulphonic acids in the cooking of wood with sulphurous acid and bisulphites. The fact that chlorine in the absence of moisture has no appreciable action on lignin (13, 22, p. 102) is fairly strong evidence against the presence of unsaturated ethylene linkages in lignin. On the other hand, a considerable number of unsaturated compounds are now known which do not add on bromine, even in the presence of catalysts.

Fuchs (7) has recently obtained some indirect evidence indicating the presence of a glucal complex in lignin.

Phenolic Nucleus

Such evidence as the similarity of lignin color reactions with those of certain phenols (8, pp. 170-171); the similarity of the ultra-violet absorption spectrum of lignin sulphonic acid solutions with that of polymerized coniferyl alcohol (9); and the presence of the glucoside, coniferin, in the cambial sap, has, as yet, no real quantitative significance. In the first place, the lignin used in every research up to the present for color reactions, has never been shown definitely to be a homogeneous chemical substance and it is not yet possible to show that the ultra-violet spectrum obtained is that of a typical coniferyl derivative, and not due to one of a number of other organic products.

Kürschner (18, p. 86) claims to have obtained up to 60% of vanillic acid by sublimation at 190 to 210° C. of a specially prepared Willstätter lignin, but these results have never been confirmed.

The most convincing evidence for the presence of phenolic nuclei in lignin is based on the results of alkali fusion of lignin preparations, catechol and protocatechuic acid, being obtained in varying yields (11, 14, 15, 19, 21). The earlier work of Heuser and coworkers (11) has recently been repeated by Freudenberg, Harder and Markert (5), who obtained a yield of only about 5% of protocatechuic acid by potash fusion of lignin. On the other hand, Rassow and Zickmann (21), by a modification of the process, obtained a yield of 15% of the crystalline acid. Such an amount might be formed readily from hydroaromatic nuclei, or even some open-chain configuration. Eugenol gives similar low yields of protocatechuic acid on alkali fusion. The results do not throw a great deal of light on the number of phenolic nuclei, if any, which may be present in the lignin molecule.

Freudenberg and Hess (6), using toluene sulphonchloride as a reagent for distinguishing between aliphatic and phenolic hydroxyl groups, conclude that one out of five hydroxyl groups in lignin is phenolic. This, according to their results, corresponds to one monohydric phenol nucleus in a molecule of molecular weight of about 800.

Tautomeric Ketomethylene Groups

The constitutional formulae for lignin proposed by Cross and Bevan (1, p. 104), Schrauth (23) and Jonas (16), all contain one or more ketomethylene groups. The assumption of the presence of such a grouping is an interesting one, as it implies the existence of both a carbonyl group and a latent ethylene linkage, which would explain why lignin appears to be unsaturated under some conditions and not under others.

Friedrich (4) claims to have obtained two tautomeric forms of soluble lignin, mutually convertible into each other. On account of the absence of criteria regarding the identity of lignin products, and the effect of traces of water on the action of halogens on lignin, this statement must be accepted with some reserve.

A Kinetic Study of the Action of Hypochlorous Acid and Chlorine Water on Lignin

The action of hypochlorous acid on ketomethylene compounds may be assumed to consist in an addition to the enolic form, and will therefore be dependent on the rate of enolization as well as on the rate of addition. The behavior of lignin, when treated with hypochlorous acid, should therefore throw considerable light on the presence of these groupings.

The action of hypochlorous acid solutions (as distinguished from that of chlorine water, hypochlorites and acidified hypochlorite solutions) on wood meal, or on lignin, has apparently not been previously investigated. Any survey of the action of hypochlorous acid on lignin must, however, take into account previous work done using chlorine water, or hypochlorites, since these react with phenols and unsaturated compounds to give mainly the same reaction products as solutions of hypochlorous acid (26).

Cross and Bevan (2) were the first to make an extensive study of the chlorination of lignin. They investigated the chlorination of jute, and, from the fact that about half of the chlorine absorbed is converted into hydrochloric acid, concluded that the reaction is one of simple substitution. This conclusion is not justified since chlorhydrin formation would give the same ratio. The following schematic relationship will illustrate this point:

$$Cl_2 + H_2O \rightleftharpoons HOCl + HCl$$

$$RH + Cl_2 \rightarrow \underset{\text{(substitution)}}{R.Cl + HCl} \qquad \Big| \qquad R.CH{:}CHR' + HOCl \rightarrow \underset{\text{(Chlorhydrin formation)}}{RCHOH.CHClR'}$$

In the action of chlorine water on wood meal at room temperature Cross and Bevan found that considerably more than half of the chlorine absorbed was changed to hydrochloric acid, indicating an appreciable amount of oxidation. Heuser and Sieber (10) studied the action of chlorine on spruce meal at room temperature and, from the ratio of chlorine to hydrochloric acid, concluded that the reaction was mainly one of oxidation. Jonas (17) studied the chlorination of Willstätter lignin at room temperature and considered that the reaction is accompanied by a partial oxidation. Nussbaum and Ebert (20) studied the action of sodium hypochlorite on sulphite pulps and found that

"bleaching" takes place in four stages. In the first, the consumption of chlorine is very rapid. If the solution is acid, a red compound is first formed which is readily oxidized. This coloration is not obtained in alkaline solution. During the second stage, the yellow impurities are gradually oxidized, while in the third stage there is a slow oxidation of the colored and colorless oxidation products. In the last stage the cellulose itself is attacked. They found the rate of bleaching to be slow in alkaline solutions and to increase in acid solutions in proportion to the square of the acidity. Between wide limits the rate of bleaching is independent of the concentration of the hypochlorite.

The nature of the reaction has therefore received little consideration beyond the general statement that both substitution and oxidation occur.

Experimental

Materials

In the preliminary experiments the action of hypochlorous acid on wood meal was studied. The use of wood meal did not however prove satisfactory, as the rate of penetration of the hypochlorous acid into the wood meal particles plays an important part in determining the rate of the reaction.

After the present investigation had been started a new method for the isolation of lignin by glycol extraction from wood was developed in this laboratory by Hibbert and Rowley (12). The lignin was purified by dissolving in 60% alcohol, reprecipitated by pouring into a large excess of water, filtered, washed, and ground while still wet, into an aqueous suspension. The concentration of lignin in the suspension was determined by filtering a known volume, drying and weighing. Methylated lignin was prepared by methylating glycol-lignin twice by the method of Urban (27).

The lignin isolated by glycol extraction is soluble in cold dilute alkali, aqueous alcohol, acetic acid, ethylene glycol, ethylene chlorhydrin, aqueous acetone, etc.

The hypochlorous acid solutions used were prepared by distillation of a solution of boric acid and bleaching powder, according to the directions given by R. L. Taylor (25). The concentration of the solutions obtained was about $M/20$. More concentrated solutions were obtained by fractional distillation. In all of the experiments, except those used for obtaining the data in Table I, the hypochlorous acid solutions obtained in this way were agitated with freshly precipitated mercuric oxide to remove traces of hydrochloric acid.

Procedure

The temperature in all of the experiments was 0° C. in order to reduce oxidation to a minimum. The rate of absorption of hypochlorous acid was determined by titration of an aliquot portion at definite time intervals.

The method, in detail, used in obtaining the data in Table I was as follows: 326.8 cc. of $M/11.415$ hypochlorous acid (equal to 572.6 cc. $M/20$ HOCl) and 139.8 cc. of distilled water were added to a one-litre three-necked flask, provided with a stirrer, and immersed in a bath of ice and water. When the

temperature of the solution was at 0° C., 200 cc. of 0.5% lignin suspension (or 200 cc. of 0.525% methylated lignin suspension, to make up for increase in weight on methylation) previously cooled to 0° C. was added.

The concentration of hypochlorous acid in the reaction mixture was 4.295×10^{-2}; the concentration of lignin was 1.5 gm. per litre and the volume of the solution was 666.6 cc.

Samples (25 cc.) were transferred at definite time intervals to an Erlenmeyer flask containing a small amount of potassium iodide and 25 cc. of $N/10$ sulphuric acid and the liberated iodine titrated with $N/10$ sodium thiosulphate. A few drops of methyl red were then added and the solution titrated with $N/10$ sodium hydroxide. The amount of hydrochloric acid formed was calculated after allowing for the alkali formed in the action of hypochlorous acid on potassium iodide (1 cc. $N/10$ sodium thiosulphate is equivalent to 0.5 cc. $N/10$ alkali).

In obtaining the data for Tables II to VI, 10-cc. samples of the reaction mixture were titrated at definite time intervals with $N/200$ sodium thiosulphate.

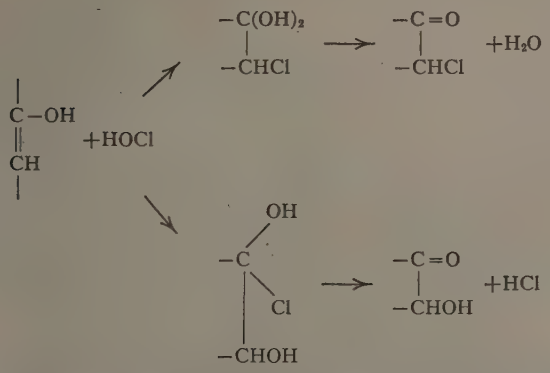
In obtaining the data for Table VII, where the amount reacting after a very short time period had to be determined, the following modified procedure was employed. A 0.25% solution of the sodium salt of lignin was prepared containing one mole of sodium hydroxide per 500 gm. of lignin; $M/200$ bromine water was also prepared. Bromine solution (10 cc.) and 188 cc. of water were added to an Erlenmeyer flask; 2 cc. of the lignin solution was transferred to a small beaker and then poured quickly into the reaction flask, washing out the beaker with the reaction mixture. At the end of six seconds an acidified solution of potassium iodide was added and the iodine liberated titrated with $N/200$ sodium thiosulphate. The experiment was repeated using varying time intervals.

Action of Hypochlorous Acid on Lignin

Examination for Ketomethylene Groups

If ketomethylene ($-CO-CH_2-$) groups are present in lignin predominantly in the keto form, and the rate of enolization is slow, they will play very little part in the reaction with hypochlorous acid, and consequently this change cannot be used to detect them. If it can be shown, however, that the fast reaction between lignin and hypochlorous acid is not due to the presence of ketomethylene groups, then it will indicate that this grouping is either not present or, if present, that it does not enolize to any appreciable extent under the given experimental conditions.

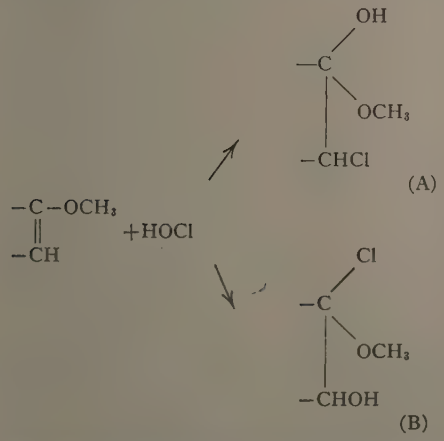
The addition of hypochlorous acid to the enolic form of a ketomethylene group may take place in either of two ways, involving a partition between two reactions, one resulting in the formation of hydrochloric acid, the other in the chlorination of the lignin.



If such a change constituted the main part of the reaction between hypochlorous acid and lignin, the amount of hydrochloric acid formed would bear a relation to the amount of hypochlorous acid absorbed, and the ratio HOCl:HCl should be fairly constant with time. As may be seen from Table I, this is actually the case. This agreement, however, appears to be only a fortuitous one. The same ratio is not obtained at lower dilutions.

The effect of methylation of the lignin on the course of the reaction with hypochlorous acid provides a more reliable method for determining whether ketomethylene groups take part in the reaction.

Schrauth (23), who includes several such groupings in his formula for lignin, considers that the enolic hydroxyl groups can be completely methylated. Assuming this for the present, let us consider the effect of methylation on the course of the reaction. It can be seen at once that the total absorption of hypochlorous acid should remain the same, while the formation of hydrochloric acid should be entirely prevented, on the assumption that no hydrolysis occurs.



The rates of absorption of hypochlorous acid by glycol-lignin, and formation of hydrochloric acid at 0° C. were determined. Glycol-lignin was then methy-

lated twice by the method of Urban (27) and the rates of absorption of hypochlorous acid by this methylated lignin, and amount of hypochloric acid formed at 0° C. were determined as before, using the same concentration of lignin and hypochlorous acid. The results are given in Table I.

The amount of hydrochloric acid formed is decreased by methylation of the lignin, but the total amount of hypochlorous acid absorbed is decreased to about the same extent, leaving the amount of hypochlorous acid absorbed without formation of hydrochloric acid practically unaffected by methylation. The results seem to indicate, assuming no hydrolysis of (A) or (B), that hypochlorous acid absorption, resulting in chlorination, is a separate and independent

TABLE I

RATE OF ABSORPTION OF HYPOCHLOROUS ACID BY GLYCOL-LIGNIN AND METHYLATED GLYCOL-LIGNIN AT 0° C. COMPARISON OF AMOUNT OF HYDROCHLORIC ACID FORMATION IN BOTH CASES

Time in min.	Cc. *M*/20 HOCl absorbed per 25 cc. reaction mixture		Cc. *M*/20 HOCl absorbed by 0.15 gm. lignin		Cc. *M*/20 HCl formed per 25 cc. reaction mixture		% HOCl changed to HCl		Cc. *M*/20 HOCl absorbed without HCl formation, per 25 cc. reaction mixture	
	Glycol-lignin	Methylated lignin	Glycol-lignin	Methylated lignin	Glycol-lignin	Methylated lignin	Glycol-lignin	Methylated lignin	Glycol-lignin	Methylated lignin
5	7.05	4.35	28.2	17.4	3.3	0.70	46.8	16.1	3.75	3.65
10	7.45	—	29.8	—	4.0	—	53.7	—	3.45	—
12.5	—	5.45	—	21.8	—	1.0	—	18.3	—	4.45
20	8.15	—	32.6	—	4.0	—	49.1	—	4.15	—
22	—	6.0	—	24.0	—	1.2	—	20.0	—	4.8
30	8.55	6.3	34.2	25.2	4.0	1.4	46.8	22.2	4.55	4.9
45	9.15	6.65	36.6	26.6	4.5	1.6	49.2	24.1	4.65	5.05
60	9.65	7.0	38.6	28.0	4.7	1.8	48.7	25.7	4.95	5.2
90	10.15	7.45	40.6	29.8	4.6	2.2	45.3	29.5	5.55	5.25
120	10.45	7.75	41.8	31.0	4.7	2.4	45.0	30.9	5.75	5.35
150	10.75	8.25	43.0	33.0	4.9	2.8	45.6	33.9	5.85	5.45
180	11.05	8.45	44.2	33.8	—	3.0	—	35.5	—	5.45

NOTE:— *Reactants used: HOCl, 4.295×10^{-2}; lignin, 1.5 gm. per litre.*

reaction from the formation of hydrochloric acid. It would seem that keto-methylene groups are either not present in lignin or, if present, do not enolize to a sufficient extent to react rapidly with hypochlorous acid.

Examination for Ethylene Linkages and Phenolic Nuclei

An investigation of the presence of ethylene linkages and phenolic nuclei in lignin was made by studying the reaction at a lower concentration ($HOCl = 10^{-2}$; lignin = 1 gm. per litre) and determining the effect of hydrochloric acid and of alkali on the reaction. It has been previously shown (26) that addition of hypochlorous acid to an ethylene linkage is catalyzed by undissociated hydrochloric acid. The velocity of such a reaction, in the presence of hydrochloric acid, is expressed by the relation

$$\frac{dx}{dt} = k\,(HCl)^2\,(HOCl) \qquad (1)$$

The velocity of any reaction in aqueous medium, such as chlorination of a phenol or phenol ether, which is dependent on the concentration of chlorine, will be expressed by the same equation, since

$$(Cl_2) = K\,(HCl)^2\,(HOCl)\,.$$

It is not possible, therefore, to distinguish between chlorination of a phenol and chlorhydrin formation by studying the effect of hydrochloric acid on the reaction. The effect of alkali on the reaction provides us however with a method of doing this.

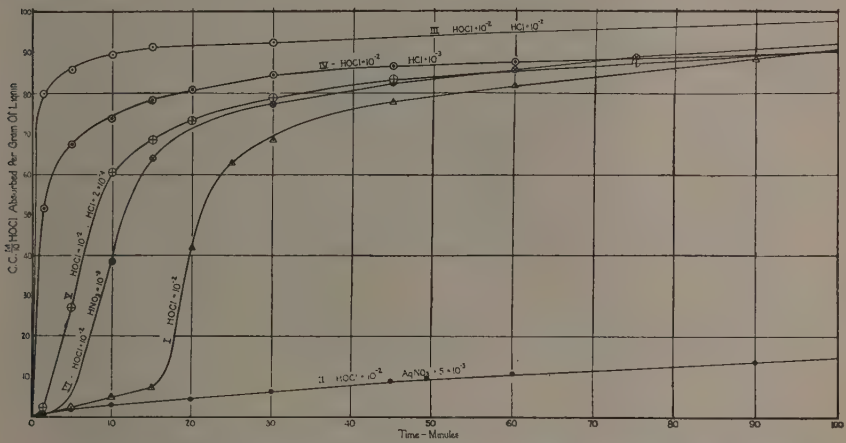


FIG. 1.—*Effect of hydrochloric and nitric acids on rate of absorption of hypochlorous acid by glycol-lignin at 0° C. HOCl = 10^{-2}; lignin = 1 gm. per litre.*

The results for the uncatalyzed reaction are given in Table II and shown graphically in Curve I, Fig. 1 and 2. The effect of *M*/100 hydrochloric acid on the reaction is shown in Table III and in Curve III, Fig. 1; and that of silver nitrate on the reaction in Table IV and Curve II, Fig. 1. The influence of *M*/1000 hydrochloric acid, *M*/5000 hydrochloric acid, and *M*/1000 nitric acid, is also included in Curves IV, V and VI, Fig. 1. As the curves are drawn to scale, the data are not given.

TABLE II

ACTION OF HYPOCHLOROUS ACID ON GLYCOL-LIGNIN AT 0° C., UNCATALYZED REACTION

Time in min.	Cc. *N*/200 $Na_2S_2O_3$ required per 10 cc. reaction mixture	Cc. *M*/400 HOCl absorbed per 10 cc. reaction mixture	Cc. *M*/10 HOCl absorbed per gram of lignin
5	39.0	1.0	2.5
10	38.0	2.0	5.0
15	37.0	3.0	7.5
20	23.2	16.8	42.0
25	14.8	25.2	63.0
30	12.6	27.4	68.5
45	8.8	31.2	78.0
60	7.3	32.7	81.75
150	2.0	38.0	95.0

NOTE:— *Reactants used: HOCl, M/100; lignin, 1 gm. per litre. No catalyst added.*

TABLE III

ACTION OF HYPOCHLOROUS ACID ON GLYCOL-LIGNIN AT 0° C.
INFLUENCE OF HYDROCHLORIC ACID

Time in min.	Cc. $N/200$ $Na_2S_2O_3$ required per 10 cc. reaction mixture	Cc. $M/400$ HOCl absorbed per 10 cc. reaction mixture	Cc. $M/10$ HOCl absorbed per gram of lignin
1.5	8.0	32.0	80.0
5	5.7	34.3	85.75
10	4.3	35.7	89.25
15	3.5	36.5	91.25
20	3.3	36.7	91.75
25	—	—	—
30	3.0	37.0	92.5

NOTE:— *Reactants used: HOCl, M/100; lignin, 1 gm. per litre; HCl, M/100.*

TABLE IV

ACTION OF HYPOCHLOROUS ACID ON GLYCOL-LIGNIN AT 0° C.
INFLUENCE OF SILVER NITRATE

Time in min.	Cc. $N/200$ $Na_2S_2O_3$ required per 10 cc. reaction mixture	Cc. $M/400$ HOCl absorbed per 10 cc. reaction mixture	Cc. $M/10$ HOCl absorbed per gram of lignin
5	39.2	0.8	2.0
10	38.8	1.2	3.0
20	38.2	1.8	4.5
30	37.4	2.6	6.3
45	36.4	3.6	9.0
60	35.7	4.3	10.75
90	34.6	5.4	13.5
120	33.6	6.4	16.0
150	32.7	7.3	18.25
180	31.9	8.1	20.25

NOTE:— *Reactants used: HOCl, M/100; lignin, 1 gm. per litre; $AgNO_3$, M/200.*

The first noticeable feature about the reaction is the autocatalytic nature when no catalyst is added. This is shown by the break in Curve I, Fig. 1. The break in the curve is accompanied by a change in the color of the lignin suspension from gray to yellowish orange. The autocatalysis is evidently due to formation of hydrochloric acid as the reaction proceeds. This is shown by the effect of silver nitrate on the reaction, and by a comparison of the effects of hydrochloric and nitric acids on the reaction (Fig. 1). The effect of added hydrochloric acid is noticeable from the beginning of the reaction (Curves III, IV and V), while nitric acid for a short period has little or no effect, evidently due to the low concentration of chlorine ion in the solution. When the critical amount of chlorine ion required is formed, the curve rises abruptly (Curve V).

The effect of excess hypochlorous acid on the reaction was determined, both for the uncatalyzed reaction, and for the reaction catalyzed by $M/100$ hydrochloric acid, by using the same concentration of hypochlorous acid as before,

and a lignin concentration of only 0.5 gm. per litre. The results are given in Tables V and VI. It may be seen that the effect of excess hypochlorous acid is not very pronounced.

TABLE V

ACTION OF HYPOCHLOROUS ACID ON GLYCOL-LIGNIN AT 0° C.
INFLUENCE OF EXCESS HYPOCHLOROUS ACID ON THE UNCATALYZED REACTION

Time in min.	Cc. *N*/200 $Na_2S_2O_3$ required per 10 cc. reaction mixture	Cc. *M*/400 HOCl absorbed per 10 cc. reaction mixture	Cc. *M*/10 HOCl absorbed per gram of lignin
5	39.9	0.1	0.5
10	39.6	0.4	2.0
15	38.9	1.1	5.5
20	36.1	3.9	19.5
25	30.6	9.4	47.0
30	28.0	12.0	60.0
35	26.8	13.2	66.0
45	25.2	14.8	74.0
60	23.7	16.3	81.5
75	22.4	17.6	88.0
90	21.9	18.1	90.5
120	20.1	19.9	99.5
240	17.2	22.8	114.0

NOTE:— *Reactants used: HOCl, M/100; lignin, 0.5 gm. per litre. No catalyst added.*

TABLE VI

ACTION OF HYPOCHLOROUS ACID ON GLYCOL-LIGNIN AT 0° C.
INFLUENCE OF EXCESS HYPOCHLOROUS ACID ON THE CATALYZED REACTION

Time in min.	Cc. *N*/200 $Na_2S_2O_3$ required per 10 cc. reaction mixture	Cc. *M*/400 HOCl absorbed per 10 cc. reaction mixture	Cc. *M*/10 HOCl absorbed per gram of lignin
1.5	22.5	17.5	87.5
5	20.8	19.2	96.0
10	19.2	20.8	104.0
15	17.9	22.1	110.5
20	17.4	22.6	113.0
30	16.5	23.5	117.5
45	15.7	24.3	121.5
60	15.0	25.0	125.0

NOTE:— *Reactants: HOCl, M/100; lignin, 0.5 gm. per litre; HCl, M/100.*

The effect of hydrochloric acid on the reaction is what might be expected if the reaction were either chlorhydrin formation or a very fast chlorine substitution. The rapidity of the reaction between lignin and a solution of the same composition as *M*/100 chlorine water (Curve III, Fig. 1) suggests that the reaction is chlorhydrin formation, chlorination of a phenol or a phenol ether, or a combination of these reactions.

A feature characteristic of the action of hypochlorous acid on phenols which is not generally characteristic of the addition of hypochlorous acid to an

ethylene linkage, or of the action of hypochlorous acid on phenol ethers, is the effect of alkali on the reaction. Soper and Smith (24) showed that the velocity of interaction between hypochlorous acid and phenols in slightly alkaline solutions increases with increasing hydroxyl ion concentration until it becomes too rapid for measurement. They also showed that if small amounts of alkali were added to the phenol on the one hand, or to the hypochlorous acid on the other, before mixing, the effect is quite different in the two cases. When sodium hypochlorite is added to the phenol the course of the reaction is quite normal, but if hypochlorous acid is added to the sodium salt of the phenol, a rapid reaction occurs between the phenol ion and hypochlorous acid, a large percentage of these reacting before equilibrium is reached. In the case of chlorhydrin formation the only effect of alkali is to decrease the concentration of free hypochlorous acid and to neutralize any hydrochloric acid formed, both of these effects tending to slow down the reaction. In the action of hypochlorous acid on phenol ethers the effect of alkali is to neutralize traces of hydrochloric acid and prevent any reaction whatever taking place.

Several experiments were carried out to determine whether the effect of alkali on the reaction between lignin and hypochlorous acid indicated a phenolic nucleus in lignin. In the case of lignin, equilibria involving ionization are apparently reached only slowly, as shown by the fact that an equivalent amount of sodium hydroxide (determined by adding acid to a solution of lignin in excess sodium hydroxide until precipitation just begins to take place) will not dissolve the lignin from an 0.25% suspension, even on standing. The solutions of lignin in very dilute alkali used in this investigation were prepared by dissolving the lignin in 60% alcohol, adding the aqueous alkali to this solution and then removing the alcohol by evaporating under reduced pressure. When hypochlorous acid is added to such a solution after dilution, no precipitation of lignin or its reaction product occurs until the reaction has proceeded for some time. It is probable then, that if the sodium salt of lignin is the sodium salt of a phenol, the part of the reaction with hypochlorous acid involving the chlorination of a phenol nucleus will be completed, especially at the relatively high concentration of $M/100$, before the free lignin can be displaced from its sodium salt by the hypochlorous acid.

The effect of alkali on the reaction between lignin and hypochlorous acid, both when the lignin is dissolved in the alkali and when the alkali is added to the hypochlorous acid before mixing, is shown graphically in Fig. 2. In obtaining the data for Curve VI, the lignin was dissolved directly in alkali; for Curves IV and V, the lignin was dissolved first in alcohol. In the case of Curve IV, there was not quite sufficient alkali to hold all of the lignin in solution. It may be seen from the figure that, in all of the cases where the lignin was dissolved in alkali before addition of the hypochlorous acid, there is a sharp rise in the curve at the beginning of the reaction, evidently due to the rapid action of hypochlorous acid on the sodium salt. This is followed by a flattening of the curve and then, if the concentration of alkali is not too high, by a second rise, probably at the point where the alkali has been neutralized by the hydrochloric acid formed, and the excess hydrochloric acid

present is equal to the critical concentration required to cause the break in the uncatalyzed reaction curve (Curve I). The addition of alkali to the hypochlorous acid before mixing does not cause any similar rise in the reaction curve at the start.

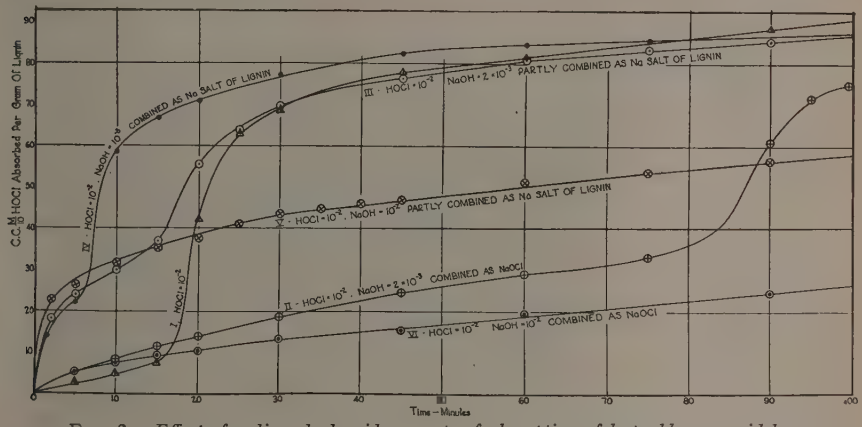
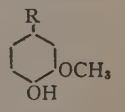

C.C. M/10 HOCl Absorbed Per Gram Of Lignin
Time—Minutes
III · HOCl · 10⁻² NaOH · 2 · 10⁻² PARTLY COMBINED AS Na SALT OF LIGNIN
V · HOCl · 10⁻² · NaOH · 10⁻² PARTLY COMBINED AS Na SALT OF LIGNIN
II · HOCl · 10⁻² · NaOH · 2 · 10⁻² COMBINED AS NaOCl
VI · HOCl · 10⁻² NaOH · 10⁻² COMBINED AS NaOCl
IV · HOCl · 10⁻² NaOH · 10⁻² COMBINED AS Na SALT OF LIGNIN
I · HOCl · 10⁻²

FIG. 2. *Effect of sodium hydroxide on rate of absorption of hypochlorous acid by glycol-lignin at 0° C. Comparison of rates when sodium hydroxide is combined with lignin and when combined with hypochlorous acid before mixing. HOCL = 10^{-2}; lignin = 1 gm. per litre.*

The amount of the rise in the curve at the beginning of the reaction when the lignin is dissolved in alkali before mixing is approximately the same for three different concentrations of alkali. This is fairly good evidence that the part of the reaction corresponding to the chlorination of a phenol is completed in the first rise; otherwise, there would be a considerable variation in the amount absorbed with the different amounts of alkali added.

The amount of hypochlorous acid absorbed in this first rise is approximately 25 cc. of a $M/10$ solution per gram of lignin, or one mole of hypochlorous acid per 400 gm. of lignin. It is difficult to determine the amount of this used up in oxidation, but a separate experiment using excess lignin indicated about 50%. If this is the case only one mole of hypochlorous acid per 800 gm. of lignin is actually absorbed in chlorinating a phenol nucleus. The phenol nucleus, most generally assumed to be present in lignin, takes up only one mole of halogen rapidly and it may therefore be concluded that only one nucleus of this type can be present in 800 gm. of lignin. Neglecting R, this is equivalent to 15.5% of such a nucleus in lignin. The presence of phenol ether nuclei in addition to this is not excluded.



The remainder of the rapid absorption in the reaction catalyzed by $M/100$ hydrochloric acid (Curve III, Fig. 1) may be due to chlorhydrin formation, chlorination of a phenol ether nucleus, or some other very rapid chlorine substitution reaction. Some of it is very probably due to chlorhydrin formation. Another investigation is planned which should show quantitatively the amount of hypochlorous acid absorption resulting in chlorhydrin formation.

Approximate figures on the amount of oxidation accompanying the chlorination were obtained for some of the runs by titration of the hydrochloric acid formed after the reaction was practically completed, using methyl orange as indicator. The solutions were too dilute to follow the course of hydrochloric acid formation throughout the reaction as was done at the higher concentration used in obtaining the data for Table I.

It was found that when no catalyst was added, about 32% of the hypochlorous acid was consumed in oxidation reactions. When the composition of the solution corresponded to $M/100$ chlorine water ($M/100$ hydrochloric acid added) about 15% was used in oxidation, while when the reaction was carried out to completion in alkaline solution 65 to 75% was used in oxidation reactions. The smaller amount of oxidation with chlorine water seems to be due to the great speed of the chlorination reaction in this case, while the greater amount of oxidation in alkaline solution is probably due to the slowing down of the chlorination reaction. The chlorination reaction is more susceptible to changes in the alkalinity or acidity of the medium than is the oxidation reaction. These facts obviously have an important bearing on the bleaching of wood pulps.

The amount of phenol nucleus in lignin, calculated from the hypochlorous acid absorption by the sodium salt of lignin, was checked by studying the action of very dilute bromine water on the sodium salt of lignin. The very rapid absorption of bromine from bromine water by all phenols is well known (3). If the sodium salt of lignin is treated with bromine water, even at concentrations as low as $M/4000$, the part of the reaction corresponding to bromination of a phenol nucleus should be completed in a few seconds. The action of $M/4000$ bromine water on the sodium salt of lignin, using the same ratio of halogen to lignin as before, was studied. The results are given in Table VII.

TABLE VII

ACTION OF BROMINE WATER ON SODIUM SALT OF LIGNIN AT 0° C.

Time in min.	Cc. $N/200$ $Na_2S_2O_3$ required per 200 cc. reaction mixture	Cc. $M/400$ Br_2 absorbed per 200 cc. reaction mixture	Cc. $M/10$ Br_2 absorbed per gram of lignin
0.1	15.0	5.0	25.0
1.0	14.4	5.6	28.0
2	13.6	6.4	32.0
5	12.5	7.5	37.5
10	11.9	8.1	40.5
20	11.0	9.0	45.0
30	10.3	9.7	48.5
45	9.5	10.5	52.5
60	9.2	10.8	54.0
90	8.9	11.1	55.5

NOTE:— *Reactants: Br_2, $M/4000$; lignin, 0.025 gm. per litre; NaOH, $M/20,000$.*

It may be seen that there is an almost instantaneous absorption corresponding to 25 cc. $M/10$ bromine per gram of lignin, the reaction then slowing

down. This first rapid absorption corresponds almost exactly to the absorption of hypochlorous acid at a concentration forty times as great as that used in the present experiment. The constancy of this first rapid absorption over such a wide range of concentration is strong confirmatory evidence for the value calculated for amount of phenol nucleus present in lignin.

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MECHANISM OF ORGANIC REACTIONS
III. THE NATURE OF THE MECHANISM OF MIGRATION OF THE ACYL RADICAL[1]

By Harold Hibbert[2] and Margaret E. Greig[3]

Abstract

The mechanism first proposed by Emil Fischer to explain the migration of acyl radicals in the case of glycerides of fatty acids receives strong confirmatory support in the isolation by the authors of a product analogous to the intermediate dioxolane derivative postulated by him. From the theoretical consideration it seemed highly probable that with any glycol monoester containing a carbonyl group of very pronounced negative polarity, the tendency to undergo cyclisation would be so pronounced as to permit of the isolation of the corresponding dioxolane ring.

It is shown that all attempts to synthesize glycol monotrichloroacetate gave, instead, the ring isomer, namely, 2-hydroxy-2′-trichloromethyl-1,3-dioxolane. The latter is quite stable at temperatures below 90° C., but at 100-110° C. decomposes into ethylene carbonate, chloroform, and a certain amount of higher-boiling, unidentified products. In presence of a trace of pyridine the decomposition into chloroform and ethylene carbonate takes place smoothly and apparently quantitatively. Similarly, when ethylene oxide is treated with dichloroacetic acid, 2-hydroxy-2′-dichloromethyl-1,3-dioxolane is produced. On the other hand, acetic and monochloroacetic acids react to give the normal half-esters. Epichlorohydrin and glycidol on treatment with trichloroacetic acid give the corresponding ring compounds, namely 2-hydroxy-2′-trichloromethyl-4-chloromethyl-1,3-dioxolane and 2-hydroxy-2′-trichloromethyl-4-hydroxymethyl-1,3-dioxolane, respectively. The last-named compound thus represents the cyclic derivative postulated by Fischer as the intermediate product formed in acyl migration occurring in the case of glycerol derivatives. Trimethylene glycol treated with trichloroacetyl chloride gives 2-hydroxy-2′-trichloromethyl-metadioxane. They are all mobile, pleasant-smelling, transparent liquids which decompose when heated at ordinary pressure.

The reaction of acetic, mono-, di-, and trichloroacetic acids on 1,2-anhydromannopyranose and 1,2-triacetyl mannopyranose is under investigation. Preliminary work appears to indicate the formation of the corresponding 1,3-dioxolane derivative of mannose in each case. The conclusion is drawn that *all organic monoesters of polyvalent alcohols, carbohydrates, polysaccharides, o-amino phenols, etc., containing a free hydroxyl group, the hydrogen atom of which is spatially in close proximity to the carbonyl group of the acyl radical, tend to pass over into a ring isomer. Theoretically, at least, there must exist in all of these cases an equilibrium between the open-chain and its corresponding cyclic isomer.*

Investigations of the structure of the monoacyl derivatives of glycerol, of carbohydrates, polysaccharides, amino phenols, etc., should permit of the isolation, in many cases, of the intermediate dioxolane or other ring concerned in the migration of the acyl radical. The existence is postulated of a definite equilibrium between the open-chain ester and the corresponding ring isomer in all monoesters of poly-alcohols in which spatial proximity of a hydroxyl to the carbonyl group exists, and the prediction is made of the probable isolation of many new cyclic isomers under investigation when appropriate consideration is given to their physical and chemical properties, such as stability, behavior towards acids, alkalies, etc.

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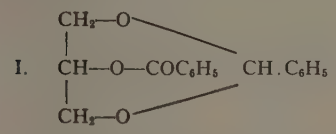
Contribution from the Industrial and Cellulose Chemistry Laboratories, McGill University, Montreal, Canada. Parts I and II were published in the Journal of the American Chemical Society, 51: 1601 and 1943 respectively, 1929.

[2] *Professor of Industrial and Cellulose Chemistry, McGill University.*

[3] *Postgraduate student, McGill University, and holder of a bursary under the National Research Council of Canada.*

Introduction

In a former communication (10) it was shown that 1,3-benzylidene glycerol 2-benzoate (I) on hydrolysis with dilute acids yielded glycerol α-benzoate (II) and not the β-benzoate (III) as was to be expected.

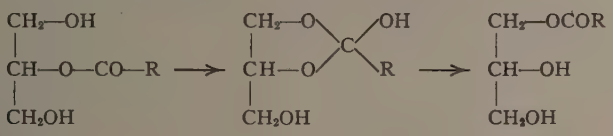


II. $CH_2OH—CHOH—CH_2O.COC_6H_5$

III. $CH_2OH—CH(OCOC_6H_5)—CH_2OH$

Evidently during the hydrolysis a migration of the benzoyl radical from the β- to the α-position had occurred. It was pointed out (10) that this phenomenon of the migration of acyl radicals in the case of the glycerol esters (6, 7) was first observed by Fischer (6) in connection with certain preliminary studies relating to the structure of fats.

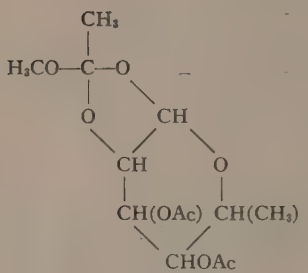
He explained the mechanism of such abnormal reactions by the assumption of the intermediate formation of a dioxolane derivative (V), formed from the β-acyl derivative (IV) by the migration of a hydrogen atom of the hydroxyl group on the carbon atom adjacent to the carbonyl group:



IV V VI

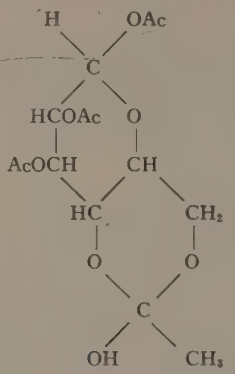
This dioxolane ring (V) then undergoes ring scission, a second migration of the hydrogen atom taking place with formation of the α-acyl derivative (VI). The same view was adopted by Hibbert and Carter (10) to explain their results.

Recently Bergmann and Carter (1) have described an ingenious synthesis of the glycerol β-esters by reducing the β-ester of the corresponding 1:3 benzylidene glycerol acetal with palladium and hydrogen. They were able to show that β-benzoyl glycerol on warming for a short time with aqueous $N/1$ hydrochloric acid does not undergo transformation into the isomeric glycerol α-benzoate. On the other hand as shown by Hibbert and Carter (10) the hydrolysis of benzylidene glycerol β-benzoate with $N/40$ HCl in 50% alcoholic solution yields the benzylidene glycerol α-benzoate. This type of (acyl) migration has recently attracted considerable attention in the field of carbohydrate chemistry as witness the recent investigations on triacetyl methyl rhamnoside (8); monoacetyl methyl mannoside (2); tetra-acetyl methyl mannoside (5); mannose tetra-acetate (4); 1,2,3,4-tetra-acetyl β-*d*-glucose (9); 2,3,4,-triacetyl β-methyl-*d*-glucoside (9); and mannose pentacetate (12); in which acetyl migration has been shown to occur with formation of a dioxolane or a meta-dioxane ring.



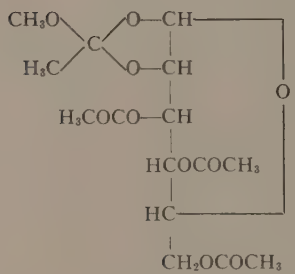
γ form of triacetyl methyl rhamnoside (8)

VII



1,2,3,4-tetra-acetyl-β-*d*-glucose (9)

VIII



IX

Tetra-acetyl methyl mannoside (3, 5)

The animated interest in the subject arises from the contrary belief held by Hudson (11) that such isomerism is the result of the formation of a new isomeric ring system. The isolation of such dioxolane or meta-dioxane intermediate isomeric ring-forms in the case of the glycerol esters, as well as in reactions involving the migration of acyl groups from oxygen to nitrogen, as in the case of the acetylated amino phenols and in other related reactions, is thus a matter of some importance, since its accomplishment would provide definite proof of the general nature of the mechanism involved in all such changes.

In order to simplify the solution of the problem of acyl migration as much as possible, it was necessary to select some product which, theoretically, should be capable of yielding a dioxolane derivative (V), possessing a relatively high degree of stability, and therefore capable of isolation.

It is evident that one way in which this might be brought about would be by increasing the polarity of the carbonyl group of the acyl radical in question, since it would then exert a much more pronounced attractive force on the hydrogen atom of the hydroxyl group in question, leading in turn to the formation of a more stable ring compound.

Acting on this idea it has been found that the monotrichloroacetates of polyhydroxy compounds show a most remarkable tendency to pass over into a dioxolane or meta-dioxane ring, a behavior which appears to be a general property of the esters of trichloroacetic acid.

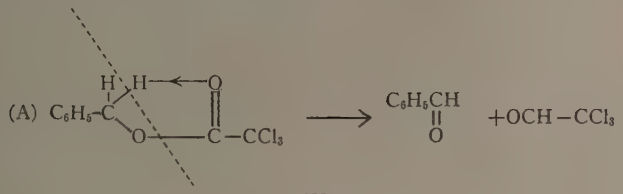
Evidence of this was first found in the changes taking place on heating benzyl-(X) and phenyl ethyl trichloroacetates (XI).

At a temperature well below that of the boiling point, the former is decomposed into benzaldehyde and chloral, the latter into phenyl acetaldehyde and chloral:

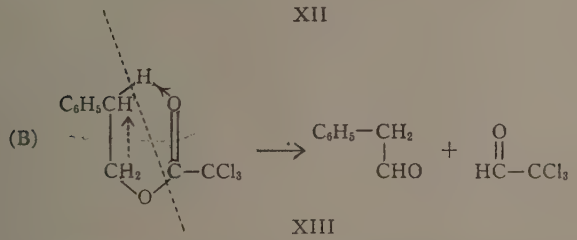
X (A) $C_6H_5CH_2OCOCCl_3 \longrightarrow C_6H_5CHO + OHC.CCl_3$

XI (B) $C_6H_5CH_2CH_2OCOCCl_3 \longrightarrow C_6H_5CH_2CHO + OHC.CCl_3$

the change presumably taking place through the intermediate formation of an unstable chelate ring, followed by ring scission:



XII

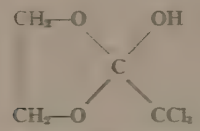


XIII

Since the corresponding benzyl and phenylethyl acetates are much more stable compounds, it is evident that the increase in the negative polarity of the carbonyl group of the trichloroacetates exerts a very pronounced action in bringing about hydrogen migration.

In the light of this preliminary information it seemed highly probable that the monotrichloroacetates of polyhydroxy compounds such as glycols, glycerols, poly-glycols, poly-ethylene glycols, carbohydrates and polysaccharides, as well as of such hydroxy derivatives as *o*-amino phenols, etc., should show a still more pronounced tendency towards ring formation.

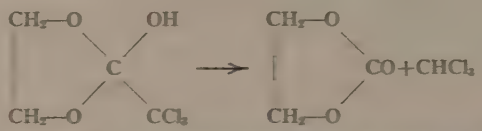
As a matter of fact the experimental evidence obtained serves to indicate that the monotrichloracetate of ethylene glycol is incapable of existence at higher temperatures than about 100-110° C. All attempts to synthesize it either by (a) action of trichloroacetic acid on ethylene oxide at room temperature; (b) action of trichloroacetyl chloride on the mono potassium salt of ethylene glycol in ether solution; and (c) interaction of ethylene glycol with trichloroacetyl chloride, yielded only the corresponding dioxolane (XIV).



2-hydroxy-2′trichloromethyl-1:3-dioxolane

XIV

The product boils very constantly and without any decomposition at 68° C. bath temperature 82° C. under a pressure of 0.025 mm. At higher temperatures and pressures it decomposes smoothly into chloroform and ethylene carbonate in presence of a trace of pyridine, a reaction which provides strong evidence as to its structure. In absence of pyridine some higher-boiling products are also formed.



It is insoluble in water, in this respect differing markedly from the normal glycol monoesters. A comparison of its boiling point with those of other mono esters also indicates that it cannot be a mono ester.

TABLE I

SUMMARY OF PROPERTIES

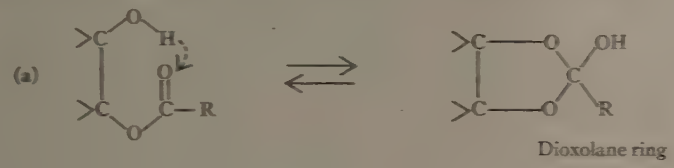
Substance	B.p., ° C.	Bath temp., ° C.	Pressure, mm.	Solubility in water
Glycol monoacetate	46 187–189	65–70 —	0.055 760	Miscible in all proportions
Glycol monochloroacetate	85 240 (with slight decomposition)	110	0.15 760	Miscible in all proportions
2-Hydroxy-2′-dichloromethyl-1,3-dioxolane	106	125	0.08	Somewhat soluble in water
2-Hydroxy-2′-trichloromethyl-1,3-dioxolane	68	82	0.025	Somewhat soluble in water
2-Hydroxy-2′-trichloromethyl-4-hydroxymethyl-1,3-dioxolane	Decomposes when distilled at 0.025 mm.			Slightly soluble in water
2-Hydroxy-2′-trichloromethyl-4-chloromethyl-1,3-dioxolane	99–101	125	0.11	Insoluble in water
2-Hydroxy-2′-trichloromethyl-*m*-dioxane	90–92	110–130	0.025	Insoluble in water

Attempts to obtain from it a methyl ether isomeric with the trichloroacetate of glycol monomethyl ether ($CH_2OCH_3-CH_2OCOCCl_3$) have up to the

present been unsuccessful, but more recent results give evidence of the possibility of effecting the desired synthesis. The results obtained provide strong experimental support for the view that the migration of acyl radicals takes place through the intermediate formation of a ring isomer.

Theoretical Deductions Relating to Acyl Migration

In the light of the above it would seem that *all organic monoesters of polyvalent alcohols: carbohydrates, polysaccharides, o-amino phenols, etc., containing a free hydroxyl group, the hydrogen atom of which is spatially in close proximity to the carbonyl group of the acyl radical tend to undergo cyclisation. Theoretically, at least, there must exist in all of these cases an equilibrium between the open-chain form and its corresponding ring isomer:*



and possibly, also, though to a less pronounced degree with a tetra glycol, diethylene glycol, etc.:

in which the formation of the less stable seven-membered ring may be postulated.

The tendency and ease of ring formation will be dependent on: a the relatively labile character of the hydrogen attached to the hydroxyl group, (b) the negative polarity of the carbonyl group in the acyl radical, and c the spatial relationship of the migratory hydrogen atom with reference to the carbonyl group.

Thus, for example, with a series of glycol mono-esters such as glycol monoacetate; glycol monochloroacetate; glycol mono-dichloroacetate and glycol mono-trichloroacetate, it may be predicted that the tendency towards cyclisa-

tion will increase from the acetate to the trichloroacetate, and experimental work in progress has shown this to be the case.

Since the vapor pressure of the ring isomers is evidently much greater than that of the open-chain form, it is to be expected that distillation of a large number of monoesters at a very low temperature and pressure (for example 10^{-5} mm.), will probably lead to the isolation in many cases of the unknown cyclic forms*.

The present experimental results also provide valuable confirmatory evidence in support of the views of Haworth and coworkers (2, 8), Freudenberg (5), Braun (3), Helferich (9) and Levene and Tipson (12) on the nature of the unusual isomerism encountered in the case of various hexose and pentose acetates.

Experimental

Action of Heat on Benzyl and Phenyl Ethyl Trichloroacetates

Benzyl Trichloroacetate

This ester was prepared by adding 25 gm. of trichloroacetylchloride slowly, with stirring, to 15 gm. of benzyl alcohol at room temperature. The reaction product, after the usual treatment, yielded 28.5 gm. of the ester, b.p. 148-149°C. (15 mm.). Analysis: Found (Stepanow method): Cl = 42.35%; calcd. for $C_9H_7O_2Cl_3$; Cl = 42.01%. Refractive Index: n_D^{14} 1.5341; $n_D^{20.8}$ 1.5312; n_D^{26} 1.5282; n_D^{31} 1.5261.

When heated under atmospheric pressure, decomposition commenced at around 220° C. Addition of water to the distillate caused it to separate into two layers. The aqueous layer contained the chloral, while the second layer was benzaldehyde, identified by its boiling point and other properties. Yield of the latter, 40%.

Phenyl Ethyl Trichloroacetate

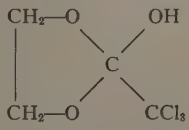
Phenyl ethyl trichloroacetate was prepared in a similar manner from phenyl ethyl alcohol, b.p. 167° C./17 mm. Analysis: Found: Cl = 39.45; calcd. for $C_{10}H_9O_2Cl_3$; Cl = 39.88%.

The ester decomposes on heating to about 240° C., the products formed being chloral, phenyl acetaldehyde and a tarry substance the nature of which was not examined.

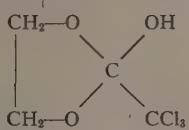
**Experiments in this connection are in progress. A number of important applications of the reactions described above, and dependent on the remarkable properties of the esters of trichloroacetic acid, at once suggest themselves. Thus it should be possible to prepare (a) the isomeric cyclic forms of glycerol monoesters, and from them the corresponding carbonates; (b) new synthetic carbonates of polyglycols, carbohydrates and polysaccharides; (c) large polyglycol- and polyethylene glycol rings; (d) vinyl derivatives of the polyglycols; (e) reaction products indicating the nature of "functional" end-groups in complex polymers; (f) the intermediate cyclic compounds which are assumed to be formed in the migration of acyl radicals from oxygen to nitrogen (amino-phenols, etc.). All of these reactions are to be investigated and the request is courteously made by the senior author for a reservation for a brief period of the application of the esters of di- and trichloracetic acid to the purposes specified. (H. H.).*

Experiments on the Attempted Synthesis of Ethylene Glycol Monotrichloroacetate

Synthesis of 2-Hydroxy-2′-Trichloromethyl-1:3-Dioxolane



Method I. Action of trichloroacetic acid on ethylene oxide. Forty grams (¼ mole) of trichloroacetic acid was dissolved in 50 cc. of dry ether, cooled to 0° C. and then added to an excess of pure ethylene oxide (40 grams) cooled to the same temperature. The mixture was allowed to stand, at room temperature, in a pressure bottle for two days. Removal of the ether and ethylene oxide under slightly reduced pressure left a pleasant-smelling oil, which was insoluble in water. When distilled under the usual water-pump vacuum (14-17 mm.) it decomposed into ethylene carbonate, chloroform and higher-boiling unidentified products. In presence of a trace of pyridine it decomposed smoothly into chloroform and ethylene carbonate. At a lower pressure (0.025 mm.), however, it was found to distil unchanged at 68° C. (bath temperature, 82° C.) Analysis: Found: Cl 51.91%; 51.63%; calcd. for $C_4H_5O_3Cl_3$: Cl = 51.2%; n_D^{25} 1.4762; n_D^{20} 1.4775. Judged by its physical and chemical properties, boiling point, solubility in solvents, and behavior towards methylating and acylating agents the product is a dioxolane derivative having the structure:



2-Hydroxy-2′-trichloromethyl-1.3-dioxolane

The same product is obtained by adding pure, freshly-distilled trichloroacetic acid to anhydrous ethylene oxide at −15° C. and allowing the contents to rise gradually to around 20° C. during the course of 5-6 hr.

Method II. Action of trichloroacetyl chloride on glycol in pyridine solution. Equimolar quantities of trichloroacetyl chloride and ethylene glycol were allowed to react at room temperature in pyridine solution. The reaction product was then heated for two hours at 60° C. After removing the pyridine under reduced pressure, it was taken up in ether, washed with water, dried with anhydrous sodium sulphate and fractionated under a pressure of about 17 mm. The only product obtained was ethylene carbonate.

Method III. Action of potassium trichloroacetate on ethylene glycol bromhydrin. The reaction was carried out in alcoholic solution. After the reaction mixture had been heated at the boiling point for two hours, the precipitated potassium bromide was removed, the alcohol distilled off and the residual oil

fractionated under reduced pressure. The principal fraction (b.p. 130-140° C./20 mm.) solidified on standing, and proved to be ethylene carbonate, m.p. 38° C. The yield was poor.

Attempts to prepare the methyl ether and an acyl derivative (benzoate, *p*-nitrobenzoate) of the ring isomer have so far proven unsuccessful.

It does not react with *p*-toluene-sulphonchloride thus indicating absence of a primary alcohol group. With ammonia in aqueous and dry ether solution it gives trichloroacetamide.

Action of Acetic, Mono-, Di-, and Trichloroacetic Acids on Ethylene Oxide, Glycidol and Epichlorhydrin

While acetic and monochloroacetic acids give the normal half-esters, dichloro- and trichloroacetic acids give the corresponding 1,3-dioxolane and meta-dioxane derivatives.

2-Hydroxy-2′-dichloromethyl-1,3-dioxolane. From dichloroacetic acid and ethylene oxide. Liquid; b.p. 106° C. at 0.08 mm., $n_D^{23} = 1.4743$. Analysis: Cl = 41.4. Calcd. for $C_4H_6O_3Cl_2$, Cl = 41.02%.

2-Hydroxy-2′-trichloromethyl-4-chloromethyl-1,3-dioxolane. From epichlorohydrin and trichloroacetic acid. Liquid; b.p. 99-101° C. at 0.11 mm., $n_D^{23} = 1.4892$. Analysis: Found Cl = 53.8. Calcd. for $C_5H_6O_3Cl_4$, Cl = 55.0%.

2-Hydroxy-2′-trichloromethyl-4-hydroxymethyl-1,3-dioxolane. From glycidol and trichloroacetic acid. Liquid; $n_D^{25} = 1.4928$. Analysis: Found Cl = 44.79% Calcd. for $C_5H_7O_4Cl_3$, Cl = 44.81%. It was not found possible to distil this substance without decomposition even at as low a pressure as 0.02 mm. This product is the first representative of the intermediate cyclic derivatives postulated by E. Fischer to explain acyl migration in the glycerol series.

2-Hydroxy-2′-trichloromethyl-metadioxane. From trimethylene glycol and trichloroacetyl chloride. Liquid; b.p. 90-92° C./0.025 mm., temp. of bath 110-130° C; $n_D^{25} = 1.4787$. Analysis: Found Cl = 48.01%. Calcd. for $C_5H_7O_3Cl_3$, Cl = 48.08%.

The products are mobile, transparent, pleasant-smelling liquids, insoluble or only slightly soluble in water. They all decompose on distillation at ordinary pressure.

The action of acetic, mono-, di- and trichloroacetic acids on 1,2-anhydro-mannopyranose and 1,2-anhydrotriacetyl-mannopyranose is under investigation. Preliminary work appears to indicate the formation in each case of the corresponding 1,3-dioxolane derivative of mannose.

Note. During the correction of the above manuscript attention was drawn to the communication by Meerwein, H. and Hinz, G. (13) in which these authors publish interesting data concerning the methylation of alcohols by means of diazomethane. They find that the action of trichloroacetic acid on ethylene oxide gives trichloroacetyl glycol as a colorless oil, b.p. 130-140° C. The action of diazomethane on this product is stated to yield a crystalline trichloroacetyl-glycol methyl ether (2-methoxy-2-trichloromethyl-1,3-dioxolane). These

experiments have been repeated by us but it has not been possible to confirm their results. In any event it is quite evident that they were concerned with products other than those mentioned in the present investigation, and at the moment no explanation can be offered as to this divergence.

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CYCLOHEXANONE-BENZIL[1]

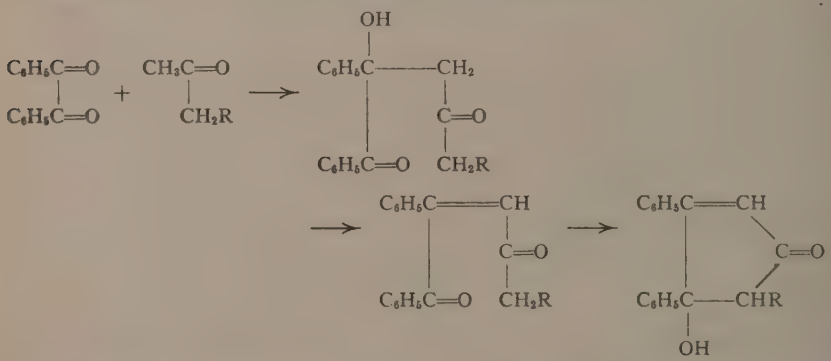
By Charles F. H. Allen[2]

Abstract

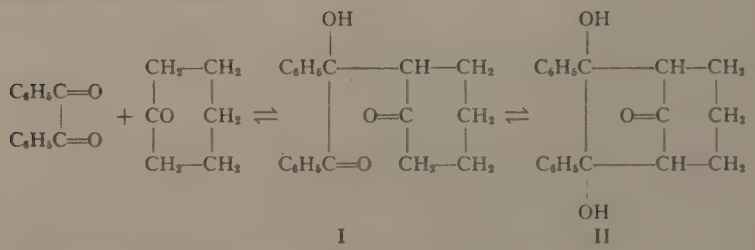
In the presence of a small amount of sodium methylate, cyclohexanone and benzil form an addition product, which is a "semicyclic" 1:4-diketone. No derivatives of a bicyclic cyclo-heptane were found. Its most conspicuous chemical property is its sensitivity to mineral acids; among the reaction products is found a diphenyltetrahydrocumarone. Although the addition product exhibits the phenomenon of halochromism, the existence of a perchlorate is doubtful, and it did not form a free radical.

Introduction

It is well known that alkyl ketones can be condensed with benzil to produce either diacylstyrenes (2, 3) or derivatives of cyclopentanone (10, 11). The latter are formed by an intramolecular addition involving a hydrogen atom in the alpha position to one of the carbonyl groups. This ring closure is so easy that it is impossible to isolate the styrene derivative unless the ketone used is so constituted that there is no available hydrogen atom (3).



Since cyclohexanone shows the typical reactions of an aliphatic ketone, it should be possible to condense it with benzil, and because it contains a hydrogen atom in the alpha position to the carbonyl group, ring closure is possible with or without loss of water.

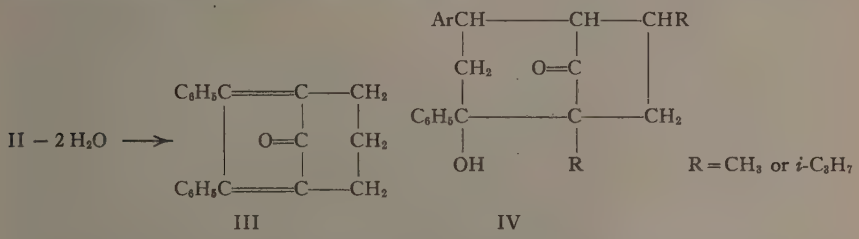


I II

[1] *Manuscript received January 31, 1931.*
Contribution from the Chemical Laboratory, McGill University, Montreal, Canada.

[2] *Assistant Professor of Chemistry, McGill University.*

The purpose of this investigation was to determine whether or not a bicyclic ring compound of type III would be formed.

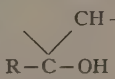


Such a ring system would be extremely interesting owing to the bridge bearing a carbonyl group between unsaturated linkages. Many of the terpenes and the endomethylene compounds (6) contain a bridged ring having alkyl groups or hydrogen but, with the exception of a very few bicyclic ketones (IV), these are the only types known with certainty at present.

Chemical Properties of Cyclohexanone-benzil

Cyclohexanone adds on very easily to benzil with formation of a compound that on analysis corresponds to an addition product composed of one molecule of each substance. According to Japp's nomenclature it would be called cyclohexanone-benzil. It forms a monoacetate and a dioxime, showing it has the structure I, and is a "semicyclic ketone" (14). It reacts with four equivalents of methyl magnesium iodide, giving two equivalents of methane, one immediately and the other slowly; the latter is probably produced as the reduced ketonic ring enolizes, since it has been well established that Grignard reagents induce enolization of ketones (8, 9).

It is not a tractable substance, very few reactions giving definite products. It is easily dissociated into its components by heat or alkaline reagents. With an equivalent amount of phenylhydrazine in ethyl acetate solution a monophenylhydrazone forms very easily in quantitative yield. If the latter is not removed but the solution refluxed, a sparingly soluble solid of high molecular weight separates in a few minutes; the structure of this was not determined.

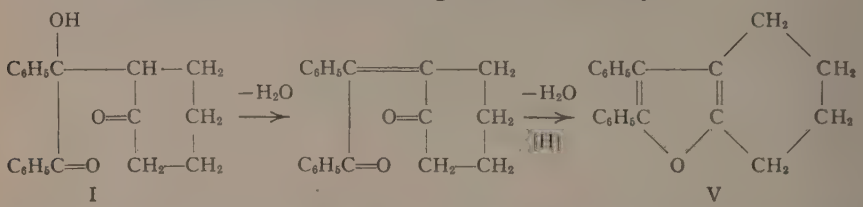
The hydroxyl group, as in many tertiary alcohols, does not form esters with most of the usual reagents; the only exception is acetyl chloride—under narrowly defined conditions half the calculated amount of a very sensitive acetate can be isolated. Garland and Reid (7) found that it was impossible to prepare acetates of certain compounds containing the grouping 
since dehydration always took place.

Cyclohexanone-benzil is extremely sensitive to mineral acids. When a drop of acid is added to a dilute solution in any solvent, (*e.g.*, acetic acid) a deep green color is produced; on adding to water the solid is precipitated and the solution becomes colorless. This is a characteristic behavior of halochromic salts, and may be represented by the equation:

$$R_3COH + HX \rightleftharpoons R_3C^+ + X^- + H_2O$$

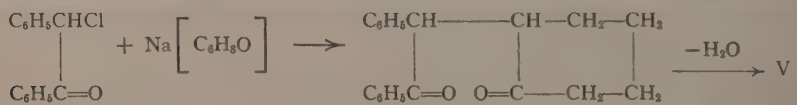
Attempts to prepare a free radical by use of Conant's reagent (5) were unsuccessful. If the solution is even moderately concentrated the color is very deep and in a few moments a thick, yellowish, sticky gum separates with the evolution of considerable heat. This gum hardens on cooling and is then very brittle. It cannot be purified, so analytical data are inconclusive. On heating in vacuo 1,2-diphenyl-3,4,5,6-tetrahydrocumarone (V) distills, and the residue in the distilling flask sets to a solid having properties like those of a paracumarone resin. No other substances could be separated.

The formation of the cumarone can be explained if it be assumed that I is dehydrated and then reduced. It is not necessary that a saturated 1:4-diketone be an intermediate product, since Lutz (13) has shown so well that unsaturated 1:4-diketones on reduction give furanes directly.



I V

Any oxidation products are lost in the resin. These same substances are obtained from the bluish-green gum left after the acetate has been isolated. The cumarone (V) was also synthesized by alkylating the sodium derivative of cyclohexanone with desyl chloride, in accordance with the following outline:



1, 2-Diphenyl-3,4,5,6-tetrahydrocumarone is relatively inactive. Naturally it shows none of the typical reactions for a carbonyl group, being unaffected by the Grignard reagent, hydroxylamine and phenylhydrazine. It cannot be reduced catalytically using Adams' catalyst (1), nor is it affected by hydrogen bromide. It loses four hydrogen atoms as hydrogen bromide with bromine; the other product is a gum. Permanganate oxidation is peculiar, in that it takes place very slowly and yields benzil. Obviously as the side chain is oxidized off, there is a breaking of C—C linkages and shifting of double bonds. It is well known that permanganate is not a reliable agent for determining the position of ethylenic linkages. Ozone does not give benzil, and nothing definite could be obtained from the gum produced.

Experimental

The Addition Product*

Finely ground benzil (12 gm.) and 6 gm. of dry cyclohexanone were mixed in a bottle of convenient size, 2 cc. of a concentrated solution of sodium methyl-

*This substance was first prepared in low yields by Mr. R. V. Elms, a senior student at Tufts College, Mass., during an investigation under the direction of the author, who takes this opportunity of acknowledging his assistance.

ate added, and the whole shaken by hand for 2-3 min. The mixture became reddish brown and warm and solidified in the course of five minutes if a bit of previously prepared addition product was added. After standing overnight the mass was loosened and shaken on a machine with a mixture of water, alcohol, ether, and a trace of acetic acid, in order to get it into a filterable condition, any lumps being crushed as much as possible. The solid was then filtered and washed, first with a small amount of alcohol and finally with ether. The yield of crude product was practically theoretical. The sodium methylate had to be freshly prepared from methyl alcohol dried with sodium, ordinary absolute alcohol not being dry enough. When the reaction took place correctly a red brown color was present all the time. If poor alcohol was used, a temporary bluish black color was noted at first, and then the mass turned brown; in the latter event the cyclohexanone was recovered by the use of sodium bisulphite but the benzil was lost. The addition is not brought about by the use of piperidine; it can be reversed by alcoholic alkaline solutions. For purification the crude addition product was recrystallized from butyl acetate in which it is very soluble when hot and almost insoluble when cold. It separated in large colorless prisms, m.p. 137° C. Analysis: Calcd. for $C_{20}H_{20}O_3$: C, 77.9; H, 6.5%; mol. wt. 308. Found: C, 77.5; H, 6.5%; mol. wt. (in acetone), 300, 306.

Cyclohexanone-benzil is very sparingly soluble in methyl and ethyl alcohols, ether and cold *n*-butyl ether, but readily dissolves in hot *n*-butyl alcohol and *n*-butyl ether, acetone, ethyl and butyl acetates, acetic acid, chloroform, acetyl chloride, acetic anhydride, benzene, nitrobenzene and pyridine. It is not affected by potassium permanganate. It slowly gives a bright yellow color with concentrated sulphuric acid, which turns red. In all solvents traces of mineral acids give a deep bluish green color. On addition of water to a glacial acetic acid solution the color disappeared and the addition product was precipitated, provided only a small amount was dissolved in a large excess of solvent originally. If at all concentrated a yellow gum separated after a few minutes. It evolved hydrobromic acid with bromine, the acid at once producing the green color and separation of yellow gum. A solution in diphenyl, heated to the boiling point (254° C.) turned yellow, the color resembling that of a solution of benzil; on cooling the color disappeared and the addition product was found to be unchanged. However, when heated alone in vacuo, it dissociated completely, the cyclohexanone distilled over first, then benzil. Five grams of addition product was heated in a small distilling flask at 10 mm.; the first portion of distillate, which boiled at 80° C. and weighed 1.5 gm. (theory, 1.6 gm.) was cyclohexanone. The temperature then rose rapidly and the last portion came over at about 250° C. as a pale yellow oil that solidified completely when seeded with a bit of benzil; yield, 3.4 gm. or 100%. There was no residue.

The substituted cyclohexanones, menthone, pulegone, and carvone, did not add to benzil under the above conditions, the components being recovered unchanged. Cyclopentanone gave an untractable pitch.

Reactions of Hydroxyl Group

We were unable to prepare a phenylurethane with phenyl isocyanate. When 5 gm. was dissolved in 5 cc. of pure phenyl isocyanate it was not changed unless heated at the boiling point for 1.5 hr. On vacuum distillation of the mixture, 0.9 gm. of diphenylurea and 2.3 gm. (68% of the theoretical) of benzil were separated from the distillate. A solution of equivalent amounts of the components in very dry benzene was unchanged after six months.

Cyclohexanone-benzil gave no methyl ether with diazomethane, nor did it form esters under any of the usual conditions with the following reagents: phthalic anhydride, acetic anhydride, benzoyl chloride, 3,5-dinitrobenzoyl chloride. Stobbe (14) could not prepare any ethers or esters with his substances of the type shown in formula IV. It gave a monoacetate with cold acetyl chloride. The finely powdered substance (5 gm.) was added to 20 cc. of acetyl chloride and the greenish mixture allowed to stand a half-hour; the solid dissolved and the solution became deep blue green. On stirring, the acetate separated as leaflets. It was filtered by suction and washed with a 2:1 ether-petroleum ether mixture; yield, 45%. The solid could not be recrystallized. It is very soluble in all organic solvents except petroleum ether and the solutions on evaporation leave bluish gums, which were distilled as described below. Analysis: sample; 0.1321, 0.0916, 0.0804 gm.; calcd. acetic acid for a monoacetate, 0.0226, 0.0157, 0.0138 gm. Found: 0.0249, 0.0150, 0.0145 gm.

The number of active hydrogen atoms in cyclohexanone-benzil was determined by the Zerewitanoff method, using methyl magnesium iodide in *n*-butyl ether; 0.1863 gm. of substance gave 27.5 cc. of methane at 765.5 mm. and 28° C.; calcd. for 2 CH_4, 27.1 cc. In the "machine" (12) the substance consumed 4.07 moles of reagent and evolved 2.25 moles of gas; one mole of gas came off almost instantly, while the second required 25 min. heating before a constant value was obtained. The solution gave a thick yellow resin which is still being investigated.

Reactions of Carbonyl Group

Cyclohexanone-benzil gave a gum with semicarbazide, but it formed a dioxime. An alcoholic hydroxylamine solution was prepared from 3 gm. of hydroxylamine hydrochloride, 6 gm. of potassium acetate and 50 cc. alcohol, the precipitated potassium chloride being removed and washed with 15 cc. of hot alcohol. To this solution was added 3 gm. of addition product and the mixture refluxed 8 hr. After standing in an open beaker for two days 1.4 gm. of rosettes of flat white needles separated and were removed; 1.8 gm. was isolated from the filtrate, making 3.2 gm. or 94% of theory. It was purified by suspending in hot chloroform, in which it is practically insoluble, and adding methyl alcohol until it dissolved. After several recrystallizations it melted at 198-9° C. with decomposition. It is very soluble in methyl alcohol. Analysis: calcd. for $C_{20}H_{21}O_3N_2$; N, 8.3. Found: N, 8.2%.

The reaction with phenylhydrazine was less conclusive; a monophenylhydrazone could be isolated by working in a particular manner, but if it

remained in the solution other reactions occurred during which an insoluble solid of high molecular weight was found, along with ammonia and aniline. Methyl phenylhydrazine and diphenylhydrazine gave only gums. Cyclohexanone-benzil (5 gm.) was dissolved in 25 cc. of hot ethyl acetate and 5 cc. of phenylhydrazine was added. In one minute 6.1 gm. (100% of theory) of fine, light yellow prisms began to separate. The solution was chilled at once, filtered, and the solid washed with alcohol. It was recrystallized by suspending in 25 cc. of hot acetone and adding pyridine until solution was completed. It separated in very fine, barely yellow prisms, m.p. 193° C. Calcd. for $C_{26}H_{26}O_2N_2$ (monophenylhydrazone); C, 78.4; H, 6.5; N, 7.0%. Found: C, 78.1; H, 6.9; N, 7.4%. It is insoluble in ether, sparingly soluble in cold chloroform, hot alcohol or acetone, very soluble in hot chloroform, benzene, ethyl acetate, and pyridine. If the phenylhydrazone was not removed, but the mixture refluxed, further reactions occurred; the solid dissolved and soon a white insoluble solid separated and caused bumping. This solid was more conveniently prepared by refluxing 1 gm. of the pure phenylhydrazone in 10 cc. of acetic acid for 1 hr.; 0.5 gm. of solid was filtered and recrystallized from pyridine or dioxane. It separated from pyridine as a fine powder, containing pyridine of crystallization, m.p. 236° C. with preliminary browning at 233° C. Analysis: found; C, 84.4, 84.3; H, 6.4, 6.5; N, 9.3, 9.4%; apparent molecular weight in camphor, 800. A sample from dioxane, m.p. 227° C., mixed with the above melted at 225-6° C. Analysis: found; C, 85.5, 85.9; H, 6.3, 6.4; N, 7.0, 7.1%; apparent molecular weight in camphor, 1600.

It was hoped that 2,4-dinitrophenylhydrazine might give a dihydrazone, but the expectations were not realized. Both with equivalent amounts and an excess of the reagent, a bright yellow compound of unknown constitution was formed. A mixture of 3 gm. of addition product, 4 gm. of 2,4-dinitrophenylhydrazine, 250 cc. of ethyl acetate and 25 cc. of benzene was refluxed 1.25 hr. and most of the solvent was distilled. After standing an hour, little yellow rosettes suddenly appeared. These were filtered and weighed 3.2 gm.; the filtrate gave an additional 0.2 gm. and much reddish oil. A mixture of 3 gm. of addition product and 2 gm. of dinitrophenylhydrazine in 35 cc. of *n*-butyl acetate containing five drops of acetic acid, after refluxing the same length of time, deposited both the yellow compound and unchanged components. After 5 hr. only 0.2 gm. of the yellow compound was found, the remainder of the product being a red oil containing small amounts of unchanged components. Solutions in pyridine gave only the oil. The yellow product recrystallized from pyridine in bright yellow leaflets, m.p. 215° C. Analysis: found; N, 10.4, 10.4, 10.3%; apparent molecular weight in camphor, 381, 392. It is insoluble in alcohol, ether, carbon tetrachloride, and acetic acid, slightly soluble in acetone, benzene and ethyl acetate, and very soluble in pyridine and dioxane.

Reactions with Acidic and Dehydrating Reagents

When to moderately concentrated solutions of cyclohexanone-benzil small amounts of hydrochloric acid were added, a deep bluish green color was produced, and in a few moments the solution became very hot and a sticky

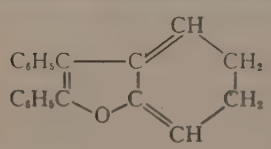
yellow gum precipitated. Although this hardened when chilled, it had no definite melting point, becoming plastic on warming. It did not crystallize from any solvent, so its exact nature was not determined. The color of the solution could be removed by adding any alkaline reagent.

All mineral acids acted in a similar manner. If the addition product was dissolved in concentrated sulphuric acid, very little heat was produced. When the red solution was poured upon ice, a flocculent purple precipitate separated; this became green on warming, and soon the same yellow plastic was noted. Anhydrous oxalic acid had no effect. In boiling xylene aluminum ethylate and cyclohexanone-benzil dissolved to a red solution, which on spontaneous evaporation left a yellow brown varnish. The acetyl chloride solution as obtained in the preparation of the acetate left a greenish gum on evaporation; the green color was readily removed by washing with an alkaline solution, and the same yellow plastic was left. Thionyl chloride acted in a similar manner.

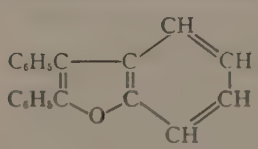
When the gum was heated in vacuo, a 20% yield of diphenyltetrahydrocumarone (V) was obtained, and the residue set to a resin. On heating to 450° C. it could be distilled, apparently without change, but it was impossible to get it into a crystalline condition.

Cyclohexanone-benzil (10 gm.) was dissolved in 20 cc. of hot glacial acetic acid and 5 cc. concentrated hydrochloric acid added. The solution turned bright green, boiled, and a yellow gum precipitated. The acid was decanted and the gum washed with water. In cold water it set to an amorphous solid which could be pulverized; it was triturated in portions in a mortar with dilute sodium hydroxide to remove traces of acid, filtered, washed well with water, and dried in a desiccator. It had no definite melting point, softening about 70° C. In a capillary tube it shrunk at about 120° C. and liquefied at 140-50°C. It dissolved readily in all the usual solvents except the alcohols and ether. When a chloroform solution was poured into methyl alcohol, or a dioxane solution into water, or the process reversed, yellow flocks separated, which acted the same as the original gum on heating. If the solution was allowed to evaporate spontaneously, a varnish was left on the walls of the containing vessel—the solvents used varied from chloroform to decalin. Although undoubtedly impure, two analyses were made, as well as several molecular weight determinations. Found: C, 87.6, 87.9; H, 6.8, 6.8%; molecular weight (mean of several determinations in freezing benzene), 2370; three different determinations in camphor gave values of 1338, 1780, 2275. Calcd. for $(C_{20}H_{18}O)_n$; C, 87.6; H, 6.6.%: $(C_{20}H_{16}O)_n$; C, 88.2; H, 5.9%; $(C_{20}H_{14}O)_n$; C, 88.8; H, 5.2%.

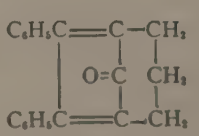
This gum might be a polymer of the tetrahydrocumarone (which is not probable, since the latter is unaffected by hydrogen bromide) or of any of the following substances which might conceivably be formed:



$C_{20}H_{16}O$



$C_{20}H_{14}O$



$C_{20}H_{16}O$

III

Isolation of a Cumarone from the Gum.

Fifty grams was distilled in vacuo, using a 125-cc. Claisen flask. At 20 mm. 13 gm. of a pale yellow oil came over at 240-60° C. (bath at 320-370° C.) As soon as a greenish fluorescence was noted in the liquid in the side arm, the distillation was stopped. The residue set, with crackling, to a solid resembling pine tree resin. The distillate did not always solidify without seed, except on prolonged standing. It was dissolved in a small amount of chloroform and poured into three volumes of methyl alcohol; if relatively free from the high boiling resin, shiny leaflets separated in a few minutes in a yield of 20%. These were always contaminated with a little of the resinous material, and required six or seven recrystallizations to get a constant melting point, *n*-propyl alcohol being the best solvent for this purpose. The yellowish color could not be removed by boiling with decolorizing carbons or infusorial earth. The pure substance is white, and crystallizes in rosettes, m.p. 120° C. Analysis: calcd. for $C_{20}H_{18}O$; C, 87.6; H, 6.8%; mol. wt. 274; found: C, 87.8, 87.9; H, 6.6, 6.7%; mol. wt. 286.

It is almost insoluble in methyl, ethyl, *i*-propyl, and *t*-butyl alcohols, hot or cold, sparingly soluble in ether and cold *n*-propyl or *n*-butyl alcohols, but very soluble in the last two alcohols when hot, butyl ether, decalin, and the other usual organic solvents. It dissolves in concentrated sulphuric acid forming a yellow solution.

Preparation of the Cumarone from Desyl Chloride and Cyclohexanone

In a flask fitted with stirrer, dropping funnel, and reflux condenser were placed 3.2 gm. of sodium wire and 25.4 gm. of dry *n*-butyl ether, and 14.6 gm. of dry cyclohexanone was slowly run in from the dropping funnel during 10 min. The mixture became warm and most of the sodium had disappeared after an hour; the last traces were removed by warming on the steam bath. The dropping funnel was removed and 33 gm. of solid desyl chloride was added directly while stirring; after half was in, the mixture became very hot and thick and was cooled externally. The remainder was then introduced and the whole heated on the steam bath for 2.5 hr. Acetic acid (10 cc.) was added and the whole steam distilled as long as any oily drops came over. There was no odor of cyclohexanone. The residual oil was taken up in chloroform, the solution dried over calcium chloride, the solvent removed, and the thick oil fractionally distilled in vacuo, collecting up to 178°, 178-235°, and above 235° C., at 10 mm. The first fraction contained unused desyl chloride and benzoic acid. The middle portion weighed 6.7 gm. and on crystallizing from *n*-propyl alcohol yielded 3 gm. of cumarone, or 12% based on the desyl chloride not recovered. That it was not formed during the distillation was shown by the actual isolation of a small amount from the residual oil from the steam distillation.

This cumarone is not affected by sodium methylate; it does not react with the Grignard reagent, nor give an oxime, phenylhydrazone, or semicarbazone. It is not changed by hydrobromic acid, but slowly decolorizes bromine in the cold with evolution of hydrogen bromide. In hot chloroform solution it

reacts rapidly, the red bromine color disappearing until about four bromine atoms have been introduced for each molecule of substance. Quantitative data were not accurate, for when an excess of bromine was used its vapors were carried over with the hydrogen bromide. In one run, 0.6396 gm. of substance was dissolved in 25 cc. of hot chloroform in an apparatus arranged to collect the gas evolved, and bromine in the same solvent added from a dropping funnel; a temporary green color changed to yellow and finally a permanent red when the bromine was in slight excess. A slow current of air was drawn through the cooled solution for 10 min., and the gases were absorbed in an excess of standard alkali. The weight calculated for 4 HBr was 0.7561 gm., but on titration it was found that 0.6899 gm. (or 91%) of HBr had been absorbed. On evaporation of the solvent a resin remained.

The cumarone could not be reduced catalytically under any of the usual conditions, using Adams' platinum black and varying the solvents from acetic acid to decalin.

It very slowly decolorized permanganate in acetone solution, forming benzil. All efforts to isolate and identify any acid except benzoic were unsuccessful. One gram of the cumarone in 30 cc. of acetone was refluxed with intermittent addition of powdered potassium permanganate until the purple color persisted for 10 min. (3 hr. were required), a little alcohol was added to remove the excess oxidizing agent, and the manganese dioxide filtered. On spontaneous evaporation 0.5 gm. of benzil, m.p. 92-3° C., was left and identified by a mixed melting point. Under the same conditions, 0.9 gm. of benzil was recovered from 1.0 gm. of pure diketone, so that the yield above represented 74% of that calculated. By alkaline extraction of the manganese dioxide and subsequent acidification, 0.2 gm. of benzoic acid was obtained and identified in the usual way.

Action of Ozone on the Cumarone.

Two-tenths of a gram of the substance was dissolved in 20 cc. of dry ethyl bromide, the solution cooled in an ice bath, and ozonized oxygen passed through for an hour. The color changes were striking. At first the solution became deep blue by transmitted, and reddish by reflected light, becoming a deep purple in five minutes. The color gradually changed to blue, then green in 40 min., after which the ozone came through; there was still some reddish reflected light. After an hour the solution was yellow, and the ozonization was stopped. Two pieces of ice were added, the nearly colorless ethyl bromide layer separated and allowed to evaporate. It left a thick oil, that dissolved easily in methyl alcohol. Nothing definite was obtained from it during a year though it was seeded with benzil, benzophenone, and the starting material.

One gram of cyclohexanone-benzil and an equal weight of aluminum ethylate in 15 cc. of xylene were refluxed for two hours, and the clear solution became a deep red. On spontaneous evaporation of the solvent a brownish varnish remained on the walls of the dish.

Half a gram of cyclohexanone-benzil was dissolved in 25 cc. of hot acetic acid, the solution chilled, and a trace of sulphuric acid added. A deep green color developed in a few minutes. After an hour, an equal volume of water

was added, the white precipitate filtered and found to be unchanged addition product, by melting point and mixed melting-point determinations.

Attempted Preparation of a Perchlorate

Following the general directions of Conant (4) a mixture of 10 cc. of 50% perchloric acid and 25 cc. of acetic anhydride was prepared. Since cyclohexanone-benzil is insoluble in ordinary ether, 1 gm. was dissolved in 50 cc. of *n*-butyl (and in a subsequent run, *n*-amyl) ether, and the above acid solution added, drop by drop, with stirring and external cooling. No solid had separated after two weeks so the procedure was repeated, using 50 cc. of acetic anhydride as solvent. The solution, deep yellow at first, soon changed through green and purple to a permanent deep pure blue. After a week, 0.1 gm. of a deep purple amorphous precipitate had formed. In a capillary tube this shrunk at 265° C. and decomposed from 270-90° C. without completely melting. It dissolved easily in benzene to form a purple solution. On titration only 40.2% of the theoretical amount of perchloric acid was found; although perchlorates often give low results on analysis for the acid, such a low figure is of doubtful value.

Attempted Preparation of a Free Radical

In an apparatus filled with carbon dioxide 1 gm. of cyclohexanone-benzil was dissolved in 75 cc. of hot glacial acetic acid and the solution cooled to the freezing point. On adding 5 cc. of concentrated hydrochloric acid the solution turned yellow, then green. When 15 cc. of a molal solution of vanadous chloride was introduced an amorphous white precipitate was formed; after 15 min. this was filtered and the clear filtrate added to 500 cc. of cold, air-free water. A white hard solid separated; both this and the precipitate above were found to be the unchanged starting material.

The operation was repeated, but with the volume of the solvent increased to 125 cc., and sulphuric acid substituted for hydrochloric. The solution was still clear after 24 hr. On pouring into water, flocks of a greenish tar separated and were removed as completely as possible. The turbid solution was allowed to stand overnight. The next day the solution was clear and a grayish green residue covered the bottom of the container. From this by use of suitable solvents was isolated 0.01 gm. of the tetrahydrocumarone (V). The rest of the residue was apparently the same yellow gum described above. Presumably the change was due to the mineral acid, as described previously, and not to any reducing action of the vanadous chloride.

Acknowledgment

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CALYCANTHINE II. THE DEGRADATION OF CALYCANTHINE TO N-METHYLTRYPTAMINE[1]

By Richard H. F. Manske[2]

Abstract

Degradation of calycanthine, by a method which has heretofore not been applied to alkaloids, namely, benzoylation with subsequent treatment with alkali, has yielded a well-defined crystalline substance, shown by analysis and synthesis to be benzoyl-N-methyltryptamine. The alternative, benzoyl-1-methyltryptamine, has also been synthesized for purpose of comparison. The bearing of the degradation product on the constitution of calycanthine is discussed. The partial structure developed for the alkaloid differs considerably from any that has been previously suggested for a natural product.

Introduction

The preparation of a nitroso derivative of calycanthine by Gordin (5) and the determination of active hydrogen by the method of Zerewitinoff together with the determination of the molecular magnitude by Späth and Stroh (12) serves to indicate rather convincingly the presence of two secondary nitrogen atoms in the molecule. Furthermore, in view of the neutral nature of the nitroso derivative, the two basic nitrogen atoms only are concerned in its formation.

The importance of the question in issue and its bearing on the constitution of the alkaloid made it desirable to prepare some characteristic derivative, and it was thought that benzoylation might yield the necessary clue in spite of Gordin's lack of success in this direction. Having failed, like Gordin, to obtain the benzoyl derivative crystalline, the true nature of the basic nitrogen atoms still remains unsolved, for it may be assumed that the action of nitrous acid as well as the Zerewitinoff reaction are as likely to produce deep-seated changes as is benzoylation. However, in view of the fact that innumerable oxidation experiments of free calycanthine under a large variety of conditions yielded inconclusive results, it was thought that oxidation of the benzoylated base might be more fruitful. The outcome was the eventual isolation of a neutral substance, which yielded analytical figures and molecular weight data agreeing with $C_{18}H_{18}ON_2$. An unusual coincidence is that the hypothetical dibenzoyl-calycanthine, $C_{36}H_{36}O_2N_4$, is just twice the molecular magnitude of the isolated compound.

The first clue to its constitution was afforded by the remarkable readiness with which it gives a pink color with Ehrlich's reagent, and the molecular magnitude as well as the empirical formula was amply confirmed by the preparation of the corresponding *m*-chlorobenzoyl and *p*-nitrobenzoyl derivatives. The fact that it is a simple N-benzoyl derivative was established by its hydrolysis with alcoholic potassium hydroxide to an oily base which on benzoylation regenerated the original substance. Finally, taking into account

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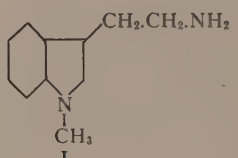
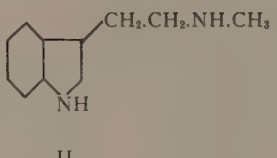
Contribution from the Department of Chemistry, Yale University, and from the National Research Laboratories, Ottawa, Canada.
[2] *Associate Research Chemist, National Research Laboratories, Ottawa.*

the fact that naturally occurring bases are almost exclusively derivatives of β-aryl-ethylamines whenever an aryl nucleus functions in the same molecule, it was logical to assume that the substance, $C_{18}H_{18}ON_2$, was a benzoyl-methyltryptamine, and having in mind a possible similarity between calycanthine and the Evodia alkaloids, attention was directed to the synthesis of benzoyl-1-methyltryptamine. The preparation of the free base (I) has recently been placed on record by Späth and Lederer (11) and benzoylation yielded the desired substance which, however, was found to be entirely different from the degradation product. The synthesis of the only logical alternative substance, namely, N-methyltryptamine (II), was therefore attempted. The method of



I



II

Decker and Becker (3), which functions so smoothly in the monomethylation of β-phenylethylamine was entirely inadequate when applied to tryptamine. The main product of the reaction was a neutral cherry-red resin, the formation of which was undoubtedly due to the condensation of the aldehyde with the 2-carbon atom of the indol nucleus.

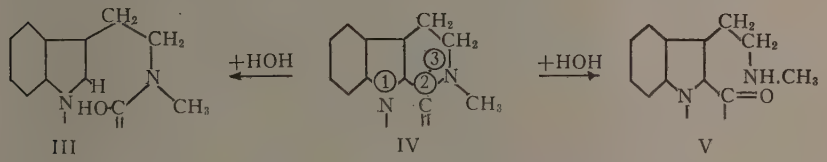
Methylation of a primary amine by means of methyl iodide generally produces a mixture, but since only the secondary base together with the unchanged primary base forms a benzoyl derivative and in view of the fact that benzoyltryptamine (4) is reputed to be readily soluble in a variety of solvents, it seemed highly probable that benzoyl-N-methyltryptamine would be readily separable from the mixture. Actually it was found that benzoylation of the mixed chloroform-soluble bases yielded the two expected products, the separation of which was accomplished practically quantitatively and without difficulty. The sparing solubility of benzoyl-N-methyltryptamine in all ordinary solvents is noteworthy, particularly in view of the much greater solubility of the unsubstituted derivative. The unusually high melting point (202° C.) is no less worthy of remark and strangely enough the melting points of the *m*-chloro- and *p*-nitro- derivatives are much lower (153° and 134° C. respectively).

The identity of the synthetic benzoyl-N-methyltryptamine with the degradation product was placed beyond doubt not only by analysis and mixed melting-point determination, but also by comparison of the characteristic rectangular crystal structure as well as the observed solubility in a variety of solvents.

At this point a digression on the significance of the color reactions which indols give with Ehrlich's reagent is desirable. It is to be pointed out that substitution in the 2- and 3-positions of the indol nucleus does not prevent the development of a pink coloration, provided that one of the substituents is of the removable type present in the Evodia alkaloids, all of which give the

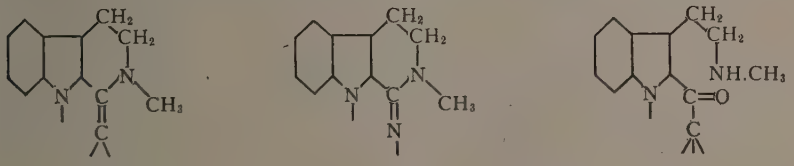
reaction after a short time of heating. On the other hand 2-keto-3-carboline (8) does not give a coloration, although condensation with methyl anthranilate (2) confers sufficient mobility on the 2-carbon atom to give the reaction*. The pink color, modified somewhat as to shade, intensity, and permanence by various substituents, is not to be confused with the greenish blue color which is given by tetrahydrocarbazol and for the formation of which an entirely different mechanism must be postulated.

Calycanthine, like evodiamine (1) forms a rather stable hydrate, and as such gives the color reaction in question much more quickly than the anhydrous base. The partial formulae (III) and (IV) are suggested to represent the transformation of the anhydrous form (IV) to the hydrate (III). Owing to the fact that a methyl group is attached to the 3-nitrogen atom, further ring



systems cannot conceivably be fused at positions (2) and (3) in analogy with rutaecarpine, and the partial formula (V) is suggested as an intermediate to account for the formation of benzoyl-N-methyltryptamine by the method already discussed. Conceivably (V) is formed from (IV) by the addition of a molecule of water to atoms (2) and (3), with the subsequent migration of the hydrogen from the resultant hydroxyl (position (2)).

Under certain conditions the benzoylated calycanthine can be transformed into the benzoyl-N-methyltryptamine by treatment with alkali and without oxidation of any kind. This observation makes it possible to extend formula (IV) to formula (VI). The alternative (VII) is almost certainly excluded,

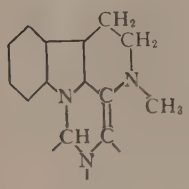


because the intermediate derived from it and corresponding to (V) is a substituted amide which, on hydrolysis with alkali would yield an indol-2-carboxylic acid, the elimination of carbon dioxide from which on oxidation or treatment with alkali is unprecedented and highly improbable. On the other hand the substance (VIII) is closely related structurally to 2-acetyl-3-phenylindol which on treatment with alkali readily loses the acetyl group

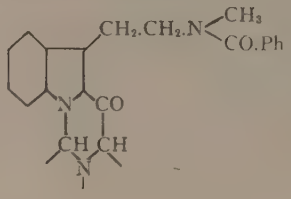
In this connection it is interesting to draw attention to the reactivity of the R-homo-keto-carboline of Jackson and Manske, (6), which occupies an intermediate position.

with the formation of 3-phenylindol (7). The elimination of an acyl group situated as in (VIII) is therefore not without precedent*.

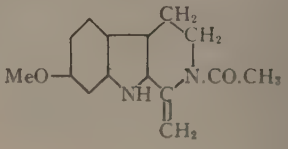
Less obvious is the mechanism by which the substituent at the pyrrol nitrogen** is replaced by a hydrogen atom. However, if the grouping, $=CH-N=$, were present as in (IX) extended from (VI), hydrolysis of the benzoylated product (X), logically leads to benzoyl-N-methyltryptamine,



IX



X



XI

together with a residue, the isolation of which is being made the subject of some work in progress.

In conclusion attention is directed to the close resemblance of the partial formula (VI) to the acetyl derivative (XI) of harmaline, the constitution of which has been established beyond doubt (10).

Experimental

Benzoylation of Calycanthine

A solution of 3 gm. of calycanthine in 60 cc. of chloroform was treated with 7 gm. of benzoyl chloride, and 14 gm. of finely powdered anhydrous potassium carbonate was added. The mixture was heated under reflux for 10 to 15 hr. at the end of which time about 100 cc. of water was added and heating under reflux continued until all the solid had dissolved. The lower chloroform layer was separated and washed thoroughly with water. A small amount of free base was extracted with a dilute solution of citric acid (about 0.4 gm. calycanthine was recovered), and the chloroform solution clarified by shaking with a small amount of potassium carbonate. The filtered solution was evaporated on a steam cone; this left the benzoylated product as an almost colorless viscous oil which still retained some chloroform and contained an appreciable quantity of benzoic anhydride.

Numerous attempts were made to obtain this product crystalline. In view of the failure to do so it is only deemed necessary to mention that among other attempts a long series of fractional precipitations from chloroform, acetone and alcohol, by the addition of ether yielded only a colorless resin which became

Further indirect evidence of a double bond as in (VI) is available from the circumstance that hydration and dehydration does not seem to alter the optical activity of calycanthine. If carbon (2) were a seat of optical activity this observation would be explicable only on the assumption of a complete asymmetrical synthesis.

**That there is a substituent here is only speculation, but in order to enable the introduction of the remainder of the molecule in a logical manner the speculation is not without basis.

friable when entirely freed of solvent. It is readily soluble in most organic solvents except ether, benzene, and petroleum ether.

When the freshly prepared substance is given a prolonged treatment with alcoholic potassium hydroxide it is hydrolyzed and calycanthine is regenerated almost quantitatively. On the other hand if it is heated for some 20 to 30 hr. at 100° C. and then hydrolyzed with alcoholic potassium hydroxide for a limited length of time (2 to 3 hr. heating on the steam bath) calycanthine is no longer regenerated. There is obtained instead a neutral product which proves to be identical with the product obtained when the crude benzoylated calycanthine is oxidized with potassium permanganate in acetone solution. The quantity obtained by the two different methods is roughly the same and amounts to about 25 to 35% of the weight of the calycanthine used.

In one experiment the benzoylated product was heated for several hours on a steam bath with alcoholic potassium hydroxide, and yielded among neutral and acidic fractions a moderate quantity of a basic substance, which is soluble in aqueous citric acid and crystallizes from alcohol in large stout rectangular plates or stout prismatic needles, melting at 235° C. It does not give a color with Ehrlich's reagent. It gives analytical figures in fair agreement with $C_{36}H_{34}O_2N_4$ or with $C_{36}H_{36}O_2N_4$. Analysis: Calcd. for $C_{36}H_{34}O_2N_4$; C, 77.98; H, 6.14; N, 10.11%; mol. wt. 554. Calcd. for $C_{36}H_{36}O_2N_4$; C, 77.70; H, 6.47; N, 10.07%; mol. wt. 556. Found: C, 77.66, 77.84; H, 6.16, 6.07, N, 10.10, 10.07%; mol. wt., 517, 496.

Work is in progress to establish the nature of this substance, but unfortunately it has proved to be difficultly accessible. Obviously the conditions for its formation are narrowly prescribed, since of the several attempts to repeat its preparation only one has been successful.

Benzoyl-N-Methyltryptamine

The benzoylated product from 3 gm. of calycanthine was freed as far as possible from chloroform by repeated evaporation with acetone, and finally dissolved in about 50 cc. of the latter solvent. Finely ground potassium permanganate (0.4 gm.) was added in small portions in the course of several hours. The mixture was agitated at frequent intervals and allowed to remain at room temperature until the pink color of the permanganate had completely faded. The mixture was filtered and the manganese dioxide was thoroughly washed with acetone. The filtrate was evaporated on a steam bath and the remaining acetone removed by repeated evaporation with alcohol. A small amount of alcoholic potassium hydroxide was added, the mixture heated for a short time, and the neutral and basic fractions precipitated by the addition of much water. The aqueous alkaline solution contained benzoic acid together with a small amount of an acid which, when dried at 100° C., was no longer soluble in aqueous sodium carbonate. To date it has not been obtained in a state of purity suitable for analysis.

The water-insoluble gum was dissolved in alcohol containing a small amount of citric acid and the neutral product precipitated by the addition of much water. A small amount of base was present in the aqueous acid solution but all attempts to obtain it in a pure condition have thus far failed.

The neutral product, which frequently crystallizes at this point, was thoroughly washed with water, dried in an oven and recrystallized from acetone with the aid of charcoal and infusorial earth. The acetone filtrate on evaporation and slow cooling yielded large colorless elongated plates, which melt at 199-200° C.* After one recrystallization the substance was obtained in brilliant crystals which melt at 202° C. It is only moderately soluble in hot chloroform or acetone, sparingly when cold and sparingly in alcohol. In ether it is practically insoluble. It gives a brilliant red color with Ehrlich's reagent. Analysis: Calcd. for $C_{18}H_{18}ON_2$; C, 77.70; H, 6.47; N, 10.07%; mol. wt. 278. Found: C, 77.14; 76.87; H, 6.58, 6.40; N, 9.90, 9.95%; mol. wt., 293, 305.

m-Chlorobenzoyl-N-methyltryptamine

The preparation of this substance follows precisely the course outlined for the unsubstituted derivative. It has been obtained by treating the crude *m*-chlorobenzoylated calycanthine with alcoholic potassium hydroxide as well as by oxidation in acetone with potassium permanganate. Owing to its much greater solubility in all solvents it is more difficult to obtain in satisfactory yield. Recrystallized from methanol it consists of short stout prisms melting sharply at 153° C. Analysis: Calcd: N, 8.96; Cl, 11.36%. Found: N, 9.11; Cl, 11.23%.

p-Nitrobenzoyl-N-methyltryptamine

This derivative was never obtained in any but very small yields. Nevertheless its formation is rather important in view of the fact that a small amount was always obtained directly from the crude *p*-nitrobenzoylation product. For the purpose of its isolation the calycanthine was heated with a mixture of *p*-nitrobenzoyl chloride and potassium carbonate as above described. The chloroform solution was desirably kept to a small volume and washed thoroughly with water. When it was then washed with dilute aqueous citric acid a pasty substance separated from the chloroform solution. The small amount of basic material removed by the citric acid solution appeared to be sufficient to retain it in the chloroform up to this point. The insoluble gum was washed with water and chloroform, dried, and crystallized from acetone. It was obtained in large golden yellow rectangular plates which give a red color with Ehrlich's reagent and melt sharply at 134° C. Analysis: Calcd. for $C_{18}H_{17}O_3N_3$; C, 66.88; H, 5.26; N, 13.00%; mol. wt. 323; Found: C, 66.78; H, 5.37; N, 13.02%; mol. wt. 324, 336.

The yield was less than 5% of the alkaloid and strangely enough it was very difficult to obtain in quantity either by oxidation or by alkali treatment of the benzoylated product.

Synthesis of Benzoyl-N-methyltryptamine

A cooled solution of 4 gm. of tryptamine in 75cc. of chloroform was treated with an excess of methyl iodide in one portion. Considerable heat was evolved and almost immediately an oil separated. The mixture was allowed to remain in ice overnight, the chloroform solution was decanted off and the gum washed

**Melting points are corrected.*

with a little chloroform. Water was added and the solution treated with a slight excess of potassium hydroxide. The liberated oily basic material was extracted with a large volume of ether-chloroform. An appreciable amount of a solid crystalline substance separated which was removed by filtering the entire mixture. It was thoroughly washed with water and with chloroform. Analysis proved it to be the quaternary salt $C_8H_7N.CH_2.CH_2N(CH_3)_3I$. It was obtained in practically colorless micaceous plates from alcohol in which it is only sparingly soluble when cold. Analysis: Calcd for $C_{13}H_{19}N_2I$; C,47.27; H,5.76; N,8.49; I, 38.48%. Found: C, 47.01; H, 5.72; N, 7.73; I, 38.39%. The same quaternary iodide may be obtained by recrystallizing the chloroform-insoluble gum (which crystallizes slowly in the course of several days) from alcohol. The yield in either case is 0.7 to 0.8 gm. from 4 gm. of tryptamine.

The ether-chloroform solution was washed with a little water, dried over sodium sulphate, evaporated until all the ether was removed and then treated with a mixture of 7 gm. of benzoyl chloride and 14 gm. of potassium carbonate. The mixture was gently heated under reflux for several hours, cooled, washed with much water, and then with a little aqueous citric acid. The resulting chloroform solution was clarified by shaking with a little sodium sulphate and evaporated somewhat. The addition of ether yielded a copious crop of colorless crystals which, when recrystallized once from hot acetone, consisted of brilliant colorless elongated plates, melting at 202° C.; yield 1.5 gm. Admixture with the substance obtained from benzoylated calycanthine showed no depression whatever in the melting point. Comparison of the crystals under a microscope failed to reveal any differences. The color reactions were the same. Analysis: Calcd. for $C_{18}H_{18}ON_2$; C, 77.70; H, 6.47; N, 10.07%. Found: C, 77.52; H, 6.44; N, 9.43%.

The ether-chloroform mother liquor, on removal of solvent and extraction with ether, yielded about 0.7 gm. benzoyl-tryptamine, m.p. 138° C. (4).

An investigation having as its object the preparation of the free base, N-methyltryptamine, and N,N-dimethyltryptamine is in progress, and it is hoped that the results will be placed on record in the near future.

Benzoyl-1-methyltryptamine

A mixture of 6 gm. of 1-methyltryptamine(10) and 8 gm. of benzoyl chloride in 100 cc. of chloroform was heated under reflux for 2 hr. with 15 gm. of finely powdered potassium carbonate. The chloroform solution was freed of acidic and basic products by washing with the appropriate solutions. The substance, however, showed no tendency to crystallize when the chloroform solution was concentrated. In view of the fact that appreciable quantities of benzoic anhydride are always formed during this method of benzoylation, the product was freed of chloroform by repeated evaporation with alcohol and then heated for a short time with alcoholic potash. The gum that was precipitated on the addition of water was thoroughly washed and then dried. The addition of ether to a concentrated alcohol solution caused the separation of pale yellow slender needles (6.5 gm.) which were filtered off, cautiously washed with ether and recrystallized from alcohol-ether solution. As thus obtained benzoyl-1-

methyltryptamine consists of colorless acicular needles melting sharply at 117° C. It is readily soluble in chloroform or acetone, moderately in alcohol, and sparingly in ether. The combined mother liquors, when freed of solvent and treated with potassium permanganate in acetone to oxidize some impurities, yielded another small amount of the substance. When the substance is heated with phthalic anhydride to 230° C. it may be recovered unchanged. It gives a brilliant red color with Ehrlich's reagent. Analysis: Calcd. for $C_{18}H_{18}ON_2$; C, 77.70; H, 6.47; N, 10.07%. Found: C, 77.59; H, 6.45; N, 10.17%.

Acknowledgment

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THE DENSITY OF CARBON DIOXIDE[1]

By D. LeB. Cooper[2] and O. Maass[3]

Abstract

The work published in a previous communication has been extended and revised. Determinations of the density of carbon dioxide are shown for five temperatures from −30° to +77° C., and over a pressure range of 760 to 250 mm. of mercury. The extrapolation of these curves to zero pressure shows a mean value for the atomic weight of carbon = 12.004 ± 0.003. A graph is included which shows the apparent molecular weight as a function of the temperature; molecular volumes have been calculated from this.

The results give P.V.T. values for CO_2 in the above temperature and pressure range, with an accuracy considered to be greater than available data.

Introduction

Preliminary measurements on the density of carbon dioxide have been published by the authors (3) in a previous communication. Since that publication the investigation has been extended, and former results checked and recalculated.

The differences in results found in this paper from the preliminary ones previously published, are due mainly to a restandardization of the constant-volume bulb under more suitable conditions, a recalibration of the thermometer PTR 3191 and the use of small corrections, which were disregarded in the previous calculations.

The change in final values at 0° C. is just appreciable, but in the results at 25° C., which were characterized as doubtful in the former paper, the change is considerable and well without the limit of error of measurement. Reasons for this deviation will be apparent later.

The method of preparation of the gas and manipulation of the apparatus are the same as before, and will not be described here. A discussion of standardizations and the use of component parts of the apparatus, disregarded for the most part in the previous publication, will be given in detail. The reader is referred to the diagram in the previous publication for the assemblage of the various sections of the apparatus.

The errors involved in the various factors determined experimentally are discussed not only from the point of view of the deviation measure of the mean, but also from the point of view of maximum error, as recommended by Birge (1) for work of this type. The probable and mean errors are not discussed.

The work, on the whole, offered considerable difficulty before consistent results were obtained, and in some cases necessitated the complete disregard of whole series of results. However no results were discarded except as a series due to determined errors, some of which are indicated in the discussion on manipulation. The latter together with the corrections involved are given

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Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Canada, with financial assistance from the National Research Council of Canada.

[2] *Postgraduate student at McGill University and holder of a fellowship under the National Research Council of Canada.*

[3] *Professor of Physical Chemistry, McGill University.*

in considerable detail not only to enable an estimate to be made of the accuracy obtained, but also to serve as a basis of reference for succeeding papers by the authors.

Introduction to Measurements

Calculations are based on the ideal gas law equation, usually written

$$PV = \frac{W}{M^1} RT,$$

where M^1 is the apparent molecular weight corresponding to a pressure P and a temperature T.

Four measurements are necessary to solve the equation for M^1; these are discussed in separate sections following.

Furthermore each standardization must be made in the same units as each other, thus if R be expressed in $\frac{\text{litre-atmospheres}}{\text{moles °C}}$, V must be expressed in litres and not in cubic centimetres; similarly if the atmosphere used in the calculation of R be the atmosphere at 45° latitude, all pressure readings must be corrected to the same standard. Other similar incidents will appear by the use of the equation of units.

In all succeeding calculations the following values were used: $G_{45} = 980.616$; litre $= 1000.027$ cm^3; 0° C. $= 273.18°$ Absolute; d_{H_2O} at 27.29° C. $= 0.996460$; $R = 0.082046$ $\frac{\text{litre-atmospheres}}{\text{moles °C}}$

Units of Equation

Standardization and Calculation of Volume

The constant volume used was constructed from a five-litre Pyrex flask, which had been aged a sufficient length of time to allow for shrinkage. The neck of the flask was drawn down and sealed to a 4-mm. bore stopcock.

The preliminary standardization of this flask was carried out in Ottawa, and is described in a previous paper. It gave the approximate value of 5442.8 ml. at 22.7° C. when under no differential pressure. After the completion of some ninety experiments the flask was removed and restandardized under more satisfactory conditions than those in Ottawa from the point of view of time available for temperature control. The method is described in detail below.

The balloon was removed from the constant-temperature bath and carefully cleaned with strong cleaning solution, followed by a mixture of zinc and hydrochloric acid, then pure nitric acid, and finally, it was washed with distilled water many times. During the washing the stopcock was allowed to remain in position, a more accurate proceeding than the former method (3) in which the volume was calculated to a point on the stem, the stopcock sealed on and the remaining portion estimated by filling it from a weight pipette. After washing, the balloon was carefully dried by blowing through it a stream of cold, pure, dried air. This left the bulb full of CO_2-free air at a known temperature and pressure. The stopcock was closed and the bulb placed on the balance pan, and allowed to remain for at least 12 hr. It was weighed on both scale pans, and the mean of the weights taken as its correct weight in air against brass. The bulb was then filled with pure distilled water.

Filling and washing the inside of the flask was conveniently carried out by partially evacuating and allowing distilled water to run in; several repetitions enabled the bulb to be filled almost to the top. At this point the bulb was connected to a good vacuum pump, and the water inside boiled under vacuum for an hour or more to remove any traces of air either in the water or adhering to the sides of the flask. The remainder was then filled with recently boiled water through clean surgical tubing.

In order to secure the desired accuracy it was necessary to know the temperature of the water to within 0.05° C. This was determined as follows. The bulb was placed in a constant-temperature bath controlled to about 0.004° C. and running somewhat above room temperature. The overflow rose into a tube of 2 mm. diameter sealed to the stopcock; readings of the meniscus were taken at intervals. When the position of the meniscus showed no change the water inside the flask was assumed to be at the temperature of that in the constant-temperature bath. This required between six and eight hours. The temperature of the bath was read on thermometer PTR 3191 which was standardized as discussed in the section on temperature.

After the bulb had reached constant temperature the stopcock was closed, and a quantity of ice thrown into the bath immediately after. One could then assume that the bulb was completely filled with water at the temperature of the constant-temperature bath, and that this temperature was known to 0.01° C. The extension tube was cleaned by suction, dried with good alcohol and ether, and finally with dry cold air. The outside of the bulb was carefully cleaned, and the whole weighed in the same manner as before. The barometric height and temperature of the balance case were read immediately after each weighing.

The standardization was repeated a second time. Again the stopcock was removed and cleaned, and the flask washed out and dried as before. The agreement between the results obtained shows that the absolute value is well within the limit of error.

The balance used was of 10-kilo capacity, with a sensitivity of 0.02 gm. with 500 gm. on each scale pan. It was solidly set up and protected from radiation by shades. It was reasonably free from temperature variations, errors from which would, in any case, be eliminated by the method of double weighing used.

The weights were a tolerance set purchased especially for standardizations. They were made of lacquered brass, and were compared against the set used to weigh the small weighing tubes, directly to 50 gm. and after that by intercomparison in the usual manner.

The following results were obtained in two complete experiments one of which is given in detail below:—

True wt. of water in vacuo, gm.	Temperature ° C.
5425.43	27.29
5425.40	27.29

Mean volume 5444.70 ml.,

if the density of water at 29.27° C. be 0.996460. The previous calibration at 22.7° C. corrected to 29.27° C. gives 5443.1.

Following are the details of a complete experiment.

	Weight of full bulb and holder, gm.	Corrected weights (gm.) to R. P.
Left pan	6076.820	6076.815
Right Pan	6076.440	6076.428
Mean		6076.62
Weight of empty bulb and holder, gm.		
Left pan		656.880
Right pan		656.910
Mean		656.89
Weight of water, uncorrected		5419.73 gm.
Weight of dry air in globe	5419.7×0.001200	$= 6.50$ gm.
Weight of air displaced by weights	$\frac{5.42}{8.39}$	$= 0.77$ gm.
True weight of water in vacuo		5425.43 gm.

Volume in ml. $= \frac{5425.43}{0.996460} = 5444.71$

Corrections to the Volume used in Calculations

In order to obtain the true volume at the temperature and pressure corresponding to those used in any single run two corrections are necessary; the first, a correction for temperature and consequent expansion or contraction of the bulb, and second, a small correction for the contraction of the bulb due to differences in pressure from the inside and the outside of the bulb.

The first correction was calculated from the equation of Keyes (7); and roughly checked by using the approximate form of the equation involving the linear coefficient of expansion of Pyrex glass (2). Both methods gave values which agreed sufficiently.

The second correction was measured directly by the method of Raleigh which was investigated and adopted by Moles and Miravalles (10), great care being taken to free the water from air. The method, which is particularly suitable in this case, showed that the function of the decrease in volume of the bulb with differences in pressure was a straight line.

The following results were obtained and are the corrections used in the calculations:

ΔP, cm.	75	50	25	10
ΔV, cc.	0.70	0.46	0.23	0.09

Measurement of Pressures

Reference to numerous tables will show that one of the pitfalls to be avoided in the construction of mercury manometers is the use of narrow-bore tubes, since it is nearly impossible to clean a tube of the desired size sufficiently to prevent distortion of the mercury meniscus. At the same time too large a tube will cause loss of accuracy in reading the meniscus by means of a telescope and cross hair. The optimum size is probably between 13 and 20 mm. diameter, in consequence of which the manometer arms were constructed of Pyrex

tubing of 15 mm. diameter. In the actual manometer used it was proved that readings could be repeated even when the two menisci were made to vary considerably in height, *i.e.* of the order of 0.1 mm.

The original form of the manometer was that more commonly used, so constructed that the two arms lay side by side. This form was tried and failure of agreement in many determinations led to the conclusion that the necessary rack motion of a cathetometer bar which was slightly off vertical would cause a considerable error in reading large differences in height. Finally the old type of manometer was replaced by one constructed as shown in the Fig. 1, which needs no rack motion of the cathetometer bar in reading original pressure. The small auxiliary arm used in reading residual pressures, placed as shown, required a small turning of the bar, but the difference in height being small this caused no appreciable error in the difference.

FIG. 1. *Construction of manometer (not drawn to scale.)*

The manometers and control reservoir were enclosed in a well-stirred water bath, the temperature of which was read immediately after each pressure reading. This was necessary to insure accurate temperature corrections. A variation of greater than 0.1° C. causes an appreciable error.

Some interest is attached to the construction of the manometer bath and its lighting system, since in a sense this controls the accuracy of reading.

The bath was made of galvanized iron. Five feet in height, with one-foot square ends, it accommodated one or two manometers without crowding. The front was pierced and two plate glass windows set in. These were free from distortion. The back of the tank was similarly fitted with a large piece of plate glass. Immediately back of this glass was placed a frame of larger size, the face of which was covered with a layer of white tissue paper. The back of the frame was grooved to allow a slide 4×12 in. to travel in a vertical plane parallel to the back of the tank. This slide was controlled from the cathetometer. Before any reading of the meniscus was attempted it was adjusted so that with the telescope (focused and free from parallax), a slight movement of the slide either up or down did not affect the apparent position of the meniscus as seen through the telescope.

Back of this slide a lighting system was fastened. This system was so built that light from eight 75-watt clear glass lamps was distributed evenly over the whole surface of the glass wall of the tank after being diffused through the thin tissue paper. A sheet metal reflector which almost enclosed the lights prevented undue lighting of the room.

With this arrangement, and proper adjustment of the slide and telescope, readings of one meniscus could be repeated to 0.002 cm. even when the cathetometer had been thrown completely out of adjustment.

The cathetometer was purchased especially for the determination. The bar of hard white brass was standardized by the Department of Geodesy and

Surveying and showed a maximum correction of 40 μ over the whole range, and 15 over the interval used in the pressure readings. The corrections were therefore negligible in all cases.

The bubble sensitivity of the level on the telescope was given as 0.1 div./30″ of arc. When received the telescope had a minimum focal length of one metre, which was too long to permit reading to 0.001 cm. with the bubble sensitivity shown. The lenses of the telescope were substituted by others which gave a minimum focal length of 13 cm. Assuming that the bubble could be set to 0.1 div., this permitted the desired accuracy.

This was tested by clamping a standard metre bar in a fixed position and repeating readings on a fixed point, throwing the cathetometer completely out of adjustment between each reading. The following typical readings show that the desired accuracy could be obtained:

Bar	95.000	95.000	97.000	97.000
Cathetometer	84.305	84.305	96.836	96.836

and the following readings of intervals:

Bar interval	90.000	90.000
Cathetometer interval	89.970	89.968

Corrections to Pressure Readings

Four corrections must be applied to all pressure readings to give the value used in the calculations. These are: (a) a correction for the density of the mercury at the temperature of the manometer bath; (b) a small correction for the residual pressure; (c) a correction for latitude; and (d) for height above sea level.

The correction for temperature was made by using the equation

$$P_0 = P_T - P_T \left[\frac{e_m t_m - e_c t_c}{1 + e_m t_m - e_c t_c} \right]$$

in which $e_m = 0.0001817$ and $e_c = 0.0000189$. Each value was calculated independently using the temperature of the manometer bath and the temperature of the cathetometer scale.

The correction for residual pressure was measured in each case and enters directly by subtraction from the original pressure.

The two final corrections which together are very small were combined into one by the use of g as determined at the Observatory. The magnitudes of the corrections were calculated from the fundamental equation

$$P_{g\,45} = P_e \frac{g_e}{g_s}$$

g at the laboratory being taken as 980.652.

Determination of Mass

The greatest advantage of this method of determining gas densities lies in the method of weighing. It will be apparent from the detailed description of the method given below that it offers considerable advantage in this particular over any of the classical methods used by Guye (6), Moles (9) and others. For this reason a considerable discussion of the method is given.

The gas was condensed into weighing bulbs as described later. These bulbs were of Pyrex glass, especially constructed for the purpose by the Corning Glass works. They were shaped as shown in Fig. 2, the tube proper of 3-mm. glass sealed to a capillary of 3-mm. wall and ½-mm. bore, in turn sealed to a tube of 4-mm. bore which enabled them to be fixed to the apparatus with ease. Each piece was leered, especially annealed, and tested with a strain finder. On the whole they were very suitable, some 100 of them from a possible 113 standing the strain without rupture, and offering a small glass surface.

FIG. 2. *Form of weighing tubes (not drawn to scale.)*

The technique of handling was as follows. After the completion of pressure readings and during the evacuation of the dead space, the outside of the tube was carefully washed with cleaning solution and distilled water, and finally wiped dry with a silk cloth free from lint. The apparatus being ready the tube was surrounded with liquid air which came into direct contact with the glass wall of the tube. Experiments showed that immersion of a weighed tube in the liquid air, and reweighing without further treatment did not alter its weight within the limit of error of experiment.

After sufficient condensation, the bulbs were sealed off near the top of the capillary, and placed in well-cleaned corked glass tubes, for storage in carbon dioxide-ether mixture until they could be weighed.

The balance used was a Sartorius with a sensitivity of 0.03 mg. per 0.01 div. with a load of 40 gm. on each scale pan. This was enclosed in a glass case one side of which had been removed and substituted by a window of thin-mesh copper gauze loosely covered with tissue paper. In the case of ruptures of the tubes this offered minimum opportunity for injury to the balance. In fact in many cases of rupture, after the scale pan had been replaced the balance point had not changed. The balance was carefully adjusted, and set up with the usual precautions and shielded from radiation.

The tubes were not placed directly on the scale pans, but were weighed using the following arrangement. On the left scale pan was soldered a small loop of thin copper wire, and the balance adjusted to zero by a similar piece soldered on the other pan. The pan rest and bearing of the left pan were removed. An air-tight copper lined wooden box was placed immediately below the balance so that a one-inch hole in the centre of the top was aligned with the hole left in the base by the removal of the pan rest bearing. This box was of one-inch oak stock 18 in. in length with square ends on 6-in. sides. It was bored, as described, at the top and coaxially with the hole in the top a three-inch hole was bored in the bottom. When in use this hole could be covered by a slide moving between the two sections of the bottom which was double. One side of the box was pierced with three equally spaced holes which admitted tightly fitting glass tubes connected to a pure dry air supply. The box was connected to the balance by a tightly fitting glass tube which fitted the hole at the top, and butted the bottom of the base immediately below the hole.

A thin glass rod hung from the loop on the bottom of the balance pan through the glass tube and into the box. This rod was terminated at its lower end by a hook on which hung a small basket made of thin pyrex rod, with a suspension of constantan wire. The whole system exclusive of the bulb weighed less than two grams and did not change in weight over long periods of time. A diagram of the arrangement is shown in Fig. 3.

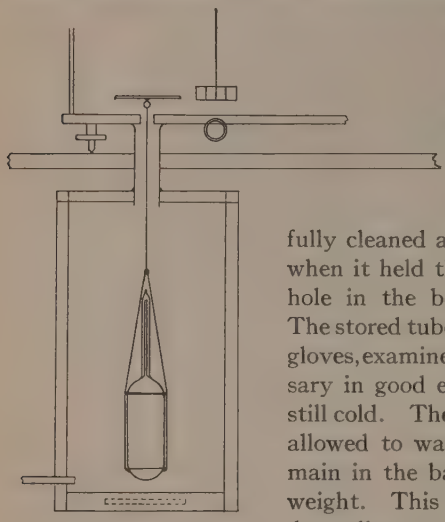

FIG. 3. *Sketch of weighing system.*

Weighings were made as follows. The suspension equipment was carefully cleaned and suspended in such a position that when it held the bulb it came over the centre of the hole in the bottom and about two inches from it. The stored tubes were taken out using silk or chamois gloves, examined for foreign material, cleaned if necessary in good ether, and placed in the basket while still cold. The dry air was turned on and the whole allowed to warm up to room temperature, and remain in the balance until it had reached constant weight. This usually required between 7 and 10 hr. depending on previous treatment of the bulb, room temperature and other variables. The bulb was weighed, usually twice to insure that it had reached true equilibrium. It was then ready to remove from the balance. To do this the slide covering the hole in the bottom was removed and a Dewar of CO_2-cooled ether inserted until it had surrounded the bulb and cooled it sufficiently to be safe for handling. The bulb was then lifted out and dipped in liquid air, and was ready to open.

The method of opening finally adopted was found to be the only suitable one. The tube was placed over a large sheet of glazed paper and a thin scratch made near the tip of the capillary. The capillary could then be easily snapped off. The difference in weight due to such a scratch was measured and found to be of the order of 0.0001 gm. Any small bits of glass were usually sucked into the tube, but in other cases were removed from the glazed paper and transferred to the balance pan. After sublimation of the carbon dioxide the bulb was blown free from the gas by a stream of dry air (free from carbon dioxide) for 20 min., carefully cleaned, treated in the same manner as before, except that it was not cooled, and allowed to remain in the balance until it had assumed constant weight. It was then weighed, removed, filled with distilled water, and weighed for the purpose of correcting to vacuo. Readings of the barometer and temperature were taken at the same time.

Owing possibly to the fact that the surface of the glass was small and the weighing conditions were good, single weights could be repeated with an accuracy beyond the limits of experimental error. Thus in one series on bulbs

much larger than those finally used the following series of weights were recorded:

Time, hr.	10	22	27	34	39	59
Weight, gm.	118.79216	118.79226	118.79288	118.79285	118.79285	118.79286

This particular tube was then put in a cooling mixture of carbon dioxide and ether, placed, while cold, in the balance case and weighed again at the end of 12 hr. The weight was 118.79293 gm. which remained constant for another 24 hr.

Corrections to Weights

The weights used for weighing the small tubes of liquid were carefully standardized against a set of laboratory standards and the departmental standards at the Department of Weights and Measures at Ottawa. Corrections for these were found to be entirely negligible. The comparison of these weights against those used in standardizing the constant-volume bulb supplied a further check.

The only other correction necessary is that needed to reduce the weights to the vacuum standard. This was done by direct measurement in all cases. After each weighing of the full tube, the tubes, having been cut open, were blown free of gas and filled with pure dry air. During the weighing of these air-filled tubes the barometer and temperature of the balance case were read and the amount of air enclosed in the tube calculated with the use of tables for the density of pure air. The volume of each individual bulb was determined immediately after the second weighing by weighing with water. Corrections for the density of the brass weights were calculated from the difference in weights in the two cases and the density of brass, which was taken as 8.39.

The intercomparison of weights in the laboratory avoided the correction necessary for the latitude and the height above sea level.

Temperature Measurement

Temperature is the final measurement necessary to discuss. It is this factor which largely controls the absolute accuracy of the results, and of the measurements made probably involves the greatest residual error.

In order to secure the desired accuracy for the density measurements, assuming for the moment that weight and volume measurements contribute nothing to the error in the final results, it was necessary to measure the temperature to within 0.02° C. This was comparatively simple at the ice point and at 25° C. but more uncertain at the other temperatures.

The arrangement at zero was as follows. The bath consisted of a well-lagged metal tank, of some 50 litres capacity filled with a mixture of salt and water, which was cooled and hand regulated by dropping in bits of crushed ice or snow at appropriate times. A Beckmann thermometer was used as an indicator, and showed that the temperature could be held constant to 0.006° C. Immediately before and after use in any single set of experiments, the Beckmann was calibrated in a Beckmann freezing-point apparatus using good distilled water. Its ice point was found to be constant over a long period of time. The movement of the stirrers in the bath caused a slight motion of the meniscus

in the mercury manometers so, immediately before making a final setting of the cathetometer, the stirring was stopped. After the reading had been recorded the temperature was read; a variation was usually not noticeable.

At 24.7° C. the arrangement was somewhat different. In this case the temperature of bath was kept to within 0.003 C., the absolute point of which was read on mercury thermometer PTR 3191. In the previous paper the calculations based on this reading were made with the use of the certificate calibration of the thermometer, which was a new one. Subsequent standardization against a standard platinum thermometer, and by the method of Richards and Yngve (11) showed that the calibration was in error by 0.10° C., sufficient to cause a considerable difference in results. Both the above standardizations yielded the same results. A slight error of unknown quantity may be present in these measurements due to periodic wandering of the ice point of the thermometer, but after two years of nearly consistent use the ice point had not changed appreciably, so error from this source is probably negligible.

The temperature at −30° C. was controlled and measured as follows. The bath liquid in this case was 95% ethyl alcohol. This was cooled to the desired temperature by carbon dioxide snow and held there by hand regulation in the same manner as at 0° C.

Temperatures were read on a platinum thermometer of 25 ohms resistance at 0° C., used with a Mueller Bridge. The set-up was sensitive to a change of 0.002° C. Standardization of the thermometer was made as follows. Resistances at 0° C. and 25.000° C. were determined. These were used for the calculation of a in the equation.

$$W = W_0(1 + at - bt^2)$$

where $b = 5.8 \times 10^{-7}$ and $a = 0.0039275$. Calculation on this basis established the absolute temperature as −30.73° C.

The bath for the high temperature isothermals was of water and in each case was controlled by electric heating. Absolute temperatures were read on standard mercury thermometers which, for added certainty, had been checked against several others.

The errors judged to be present in the absolute temperatures are shown in the section on results.

Results

The results are shown in Tables I to V. The last columns of the tables show the calculated apparent molecular weight from the equation

$$M^1 = \frac{WRT}{PV}$$

in which M^1 is the apparent molecular weight, and the other symbols have their usual significance.

Calculations were made as shown in the previous publication. In addition corrections were made to pressures for latitude and height above sea level, and the volume was corrected for pressure differences. From recent measurements made by Moles (9) and coworkers it appears that any corrections for the adsorption of carbon dioxide on the walls of the constant-volume bulbs are negligible. This is confirmed by the nature of the results obtained.

TABLE I

RESULTS OF EXPERIMENTS

Pressure, cm.	Temperature, ° A.	Volume cc.	Weight, gm.	$M^1 = \frac{WRT}{PV}$
75.427	297.61	5444.6	9.7851	44.216
72.772	297.63		9.4385	44.209
72.366	297.66		9.3827	44.198
71.168	297.63		9.2274	44.194
69.366	297.60		9.9956	44.199
68.403	297.61		8.8689	44.191
50.683	297.60	5444.3	6.5631	44.138
48.038	297.49		6.2233	44.141
48.993	297.49		6.3474	44.144
47.469	297.51		6.1486	44.136
48.477	297.52		6.2801	44.146
24.908	297.61	5444.1	3.2211	44.081
24.610	297.62		3.1824	44.081
24.811	297.64		3.2070	44.064
25.367	297.59		3.2802	44.074
25.294	297.61		3.2706	44.076
25.083	297.61		3.2436	44.080
25.016	297.44	5444.0	3.2354	44.062
25.202	297.44		3.2597	44.066
22.698	297.45		2.9365	44.077
22.325	297.45		2.8882	44.077

TABLE II

RESULTS OF EXPERIMENTS

Pressure, cm.	Temperature, ° A.	Volume cc.	Weight, gm.	$M^1 = \frac{WRT}{PV}$
71.768	273.11	5443.3	10.1569	44.277
70.341	273.18		9.9530	44.279
69.693	273.18		9.8618	44.281
71.609	273.04		10.1370	44.278
70.166	273.18		9.9267	44.272
50.065	273.04	5443.0	7.0721	44.188
47.962	273.18		6.7727	44.192
49.828	273.11		7.0379	44.193
50.482	273.01		7.1328	44.191
50.051	273.11		7.0693	44.192
26.129	273.04	5442.8	3.6851	44.117
24.029	273.11		3.3853	44.082
24.333	273.11		3.4306	44.113

TABLE III

RESULTS OF EXPERIMENTS

Pressure, cm.	Temperature, ° A.	Volume cc.	Weight, gm.	$M^1 = \frac{WRT}{PV}$
70.726	242.45	5441.4	11.3005	44.393
70.986	242.45		11.3430	44.396
44.428	242.45	5441.2	7.0748	44.246
45.791	242.45		7.2937	44.256
25.229	242.45	5440.9	4.0087	44.151
26.624	242.45		4.2317	44.166

TABLE IV

RESULTS OF EXPERIMENTS

Pressure, cm.	Temperature, ° A.	Volume cc.	Weight, gm.	$M^1 = \frac{WRT}{PV}$
68.689	322.29	5445.8	8.2187	44.156
67.814	322.30	5445.8	8.1133	44.152
68.885	322.53	5445.8	8.2377	44.163
69.686	322.54	5445.8	8.3333	44.163

TABLE V

RESULTS OF EXPERIMENTS

Pressure, cm.	Temperature, ° A.	Volume cc.	Weight, gm.	$M^1 = \frac{WRT}{PV}$
69.203	350.57	5447.3	7.6083	44.117
69.256	350.64	5447.3	7.6119	44.115
69.011	350.58	5447.3	7.5881	44.127

Reference to each section on the discussion of apparatus will show that, of the four measurements necessary, the following deviations may be assigned to each one.

Temperature of gas	−30° C.	±0.025
Temperature of gas	0° C.	±0.005
Temperature of gas	25° C.	±0.01
Temperature of gas	50° C.	±0.03
Temperature of gas	75° C.	±0.02
Pressure of gas		0.006 cm.
Volume		0.10 ml.
Weight.		0.0003 gm.

The value assigned to the error in mass is probably a maximum one, and in many cases must have been less. The final method adopted for opening the tubes does not allow of any accurate guess of the amount of deviation contributed by this factor, but the above is probably a very generous one.

Since the four measurements enter the equation directly, maximum errors in the apparent molecular weights may be calculated. These are found to vary at 25° C. and 0° C. from 0.015% at high pressures to 0.035% at the lowest pressure. Examination of the tables shows this to be the maximum difference between any observation and the mean. Of course the comparison must be made at the same pressures, and this is done by a method described below.

Inspection of the general form of curve obtained shows that these are straight lines to within the limits of experimental accuracy, and may be represented by the general equation

$$M^1 = aP + Mo.$$

This permits a general averaging of results on any isothermal according to the following method. The mean of the pressures and the corresponding molecular weight obtained at each gives a true value for the molecular weight measured at the mean value of the pressures. This value is plotted and the equation for the straight line to any tentative value for the theoretical molecular weight of carbon dioxide is obtained. The value of the molecular weight of any point near the mean is then corrected to its value at the mean value of the pressure. Table VI below gives an example of how the apparent molecular weight is obtained for a mean pressure. The data are taken from Table I.

TABLE VI

CALCULATION OF THE DEVIATION OF THE MEAN

Pressures read, cm.	Molecular weights calculated from $M^1 = \frac{WRT}{PV}$	Correction to mean	Value of M^1 at mean pressure	Deviations from mean M^1
75.427	44.216	−0.0107	44.2053	+0.0042
72.772	44.209	−0.0033	44.2056	+0.0045
72.366	44.198	−0.0022	44.1958	−0.0053
71.168	44.194	+0.0012	44.1952	−0.0059
69.366	44.199	+0.0059	44.2049	+0.0038
68.403	44.191	+0.0089	44.1998	−0.0013

Mean pressure, 71.5836; mean molecular weight, 44.2011; deviation of mean, 0.0038%.

TABLE VII

Mean pressure, cm.	Mean M^1	Temperature, ° A.	Deviation of mean average deviation, %
71.5836	44.2011	297	0.0038
48.7320	44.1410	297	0.0038
24.5314	44.0738	297	0.0050
70.7154	44 2774	293	0.0043
49 6776	44 1912	293	0.0036
24.8303	44.1040	293	0.017
70.8560	44.3945	242	0.0014
45.1095	44.2510	242	0.0041
25.9265	44.1585	242	0.0062
68.7685	44.1585	322	0.0036
69.1566	44.1196	350	0.0063

The last column in Table VI gives the deviations from the mean value, which makes it possible to calculate the deviation of the mean in the usual manner (5). The deviation of the mean in this case is 0.0038%. All the data have been treated in the same manner and are tabulated in Table VII.

Discussion of Results

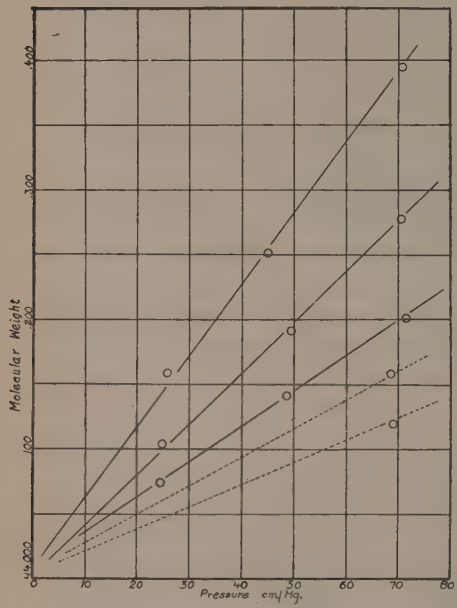

FIG. 4. *Graphs of all results. Plotted by use of the mean values.*

The results are of interest in two ways; first with regard to an equation of state, and secondly with regard to the atomic weight of carbon as given by the density measurements.

The isothermals at 25°, 0°, and −30° C. are found to be straight lines within the limit of experimental accuracy obtained (see Fig. 4). They may be represented by

$$M^1 = aP + Mo.$$

The constants a, and M_o are calculated as follows. Weights, calculated from the deviation measures, were assigned to each of the three points concerned in a line. Due to the larger number of determinations made at the low pressure at 25° C. the three points are almost equally weighted, the other extreme being the −30° C. isotherm where two determinations at each pressure means that the two higher pressures practically determine the slope of the line. The values of a and M_o are given in the following table:—

TABLE VIII

VALUES OF a AND Mo

Temperature °C.	a	Mo
25	2.706×10^{-3}	44.0083
0	3.907×10^{-3}	44.0001
−30	5.446×10^{-3}	44.0079

From the values of Mo the atomic weight of carbon has the values 12.0083, 12.0001, 12.0079, giving a mean of 12.005. Since the −30° C. isotherm has the greatest calculated error it should not be given the same weight as the others and hence 12.004 would be a better estimate from the results obtained.

With an estimate of something better than 0.01% accuracy in the determinations of *Mo* from all the data, this gives an accuracy of 0.03% in the atomic weight of carbon which can therefore be written as 12.004 ± 0.003. This agrees with the accepted estimated value determined in various ways. No claim is intended with regard to greater weight being attached to the result here obtained.

It may be pointed out that no theoretical equation was used to determine *Mo* which was simply the extrapolated value at zero pressure of the experimental results, the justification of the straight line extrapolation being given by the same experiments. With a 50-litre flask and change in manometer liquid, experiments will be carried out by means of which tenfold accuracy will be attained. This is pointed out here because the slopes of the isothermals here found will then be used with adequate accuracy for the short range of pressure extrapolation which will then be made.

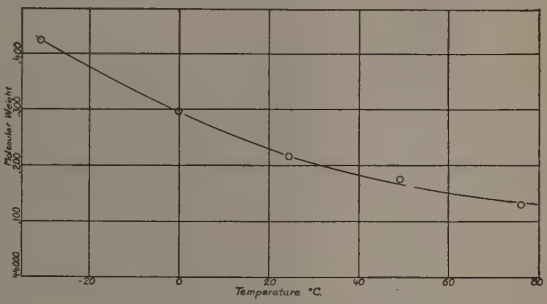

FIG. 5. *Variation of apparent molecular weight with temperature. P is constant and equal to one atmosphere.*

The values of M^1, the apparent molecular weight, and the volume of a gram mole at 76 cm. pressure over a temperature range are given in the following table. These have been obtained from the graph shown in Fig. 5 where the experimental results have been plotted.

TABLE IX

APPARENT MOLECULAR WEIGHT AND VOLUME OF A GRAM MOLE OVER A TEMPERATURE RANGE

Temperature °A	M^1	V (observed), litres
243.18	44.422	19.764
273.18	44.295	22.266
293.18	44.232	23.930
323.18	44.167	26.418
343.18	44.138	28.071

A discussion of results in connection with a gas law (8) is reserved for a later paper to follow a paper on the viscosity (12) of carbon dioxide, the data of which is required for this discussion.

Acknowledgments

The authors express their appreciation to Professor James Weir, of the Department of Geodesy and Surveying, who assisted greatly by his painstaking calibration of the cathetometer bar.

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THE PHOTO-ELECTRIC CELL AND ELECTRIC CLOCK AS A MEANS OF RECORDING THE DAILY HOURS OF BRIGHT SUNSHINE[1]

By Wallace A. Thomson[2]

Abstract

A method is presented whereby an accurate record of the daily hours of bright sunshine is made by an electric clock, controlled, through a relay, by the intensity of the light incident upon the cathode of a suitably exposed photo-electric cell.

Introduction

Continuous daily records of the hours of bright sunshine have been made by many of the more important meteorological stations in all parts of the world for the past thirty or more years, and the use of these records in various lines of scientific research is daily becoming more general.

Previous Work and Methods

One of the most common methods of obtaining these results is that by which the record is burned on a card by the solar rays concentrated by a glass sphere. Typical apparatus of this sort is the Campbell-Stokes instrument. If the instructions outlined in the Meteorological Observer's Handbook regarding the exposure, manipulation and interpretation of the card, are carefully followed, this method will give fairly satisfactory results, but there are some difficulties that cannot be readily overcome. The most apparent of these arises in the proper interpretation of the card on a day when the sun has been intermittently obscured by numerous small but dense cumulus clouds. Days such as this are very common during certain portions of the year. The cards reproduced in Fig. 1 *A* and *B* illustrate this point very clearly. The burned spots must be considered collectively and an estimate made of the total hours of bright sunshine. Such an estimate could scarcely be considered more accurate than to the nearest hour, and when a large number of more or less similar cards appear in any year, the error is liable to be considerable.

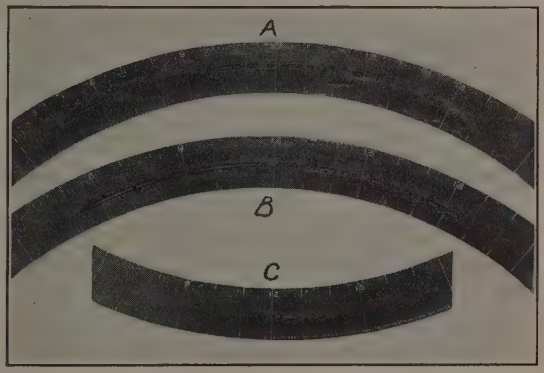

Fig. 1. *Cards from E. R. Watts Sunshine Recorder. A and B are typical records for summer days; C was exposed January 6, 1931.*

[1] *Manuscript received January 30, 1931.*
Contribution from the Physics Department, Manitoba Agricultural College, Winnipeg, Manitoba.

[2] *Lecturer in Physics, Manitoba Agricultural College.*

Another difficulty with this method arises from the fact that the burning does not start until some time after the sun has risen and stops a considerable time before the sun reaches the western horizon. There may be some who will consider this condition to be normal, but that will probably depend somewhat upon the actual use that is to be made of the records.

Scope of Present Work

With the object of attempting to overcome these difficulties the writer has made use of a photo-electric cell, a relay, and an electric clock arranged as illustrated in Fig. 2. This diagram shows one of the simplest circuits in use for the purpose of controlling an operation by allowing light of a certain intensity to fall upon the cathode of the photo-electric cell. In this instance the photo-electric cell was so exposed that the instant the direct sunlight fell upon it, the electric clock was automatically started by the action of the relay, which also stopped the clock just as promptly when the sun disappeared behind a cloud or below the horizon. It is then necessary only to read the time on the clock at the end of each day, when the number of hours it has been running since last observed is a record of the number of hours of bright sunshine during that period of time.

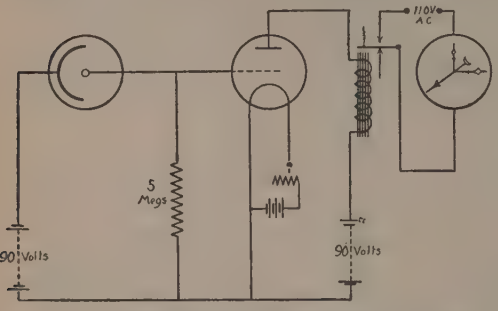

FIG. 2. *Diagram showing connections of photo-electric cell, relay and electric clock.*

Apparatus Employed

The photo-electric cell in use, which is a "Visitron" type 75*A* gas-filled cell, was mounted upright in a bakelite socket. This was fastened tightly to a wooden block about four inches square and one inch thick. The cell was then enclosed in a metal box about four inches square and six inches high. A ground glass window about two inches square was made in one side of the box at such a height that the light entering the box through this window fell directly upon the cathode of the cell. This ground glass window was rendered light proof, except for three parallel vertical slits about one inch long and one sixteenth of an inch wide, placed about a half-inch apart. A glass prism was mounted outside the window and covering the centre slit so as to reflect the sun's rays on to the cell when the sun's position was such that they would not enter through either of the other slits. This box was then placed on the roof of the building and the two lead wires brought to the laboratory below.

Careful adjustment of the amount of light incident upon the cathode of the cell was found necessary to bring its effect within the range of adjustment of the relay. The relay was then adjusted so as to start the clock when the sun's rays reached the desired intensity.

The clock is a standard type, made to operate on 110 volts A.C. 60 cycles, and automatically starts and stops when the current is turned on or off. It is provided with a large second hand, which makes it possible to tell at a glance whether or not the clock is running.

A standard telegraph relay of a commercial type was used with fair satisfaction. Some difficulty was experienced with this instrument owing to coarseness of adjustment. A much more sensitive and freely acting relay would be desirable.

The single thermionic valve used for amplification was a "Radiotron" model *UX*201*A* operated at a plate voltage of 90 and with a filament voltage of five.

Results

The following table shows a comparison between the record of the hours of bright sunshine made by the photo-electric cell and electric clock (column *A*) and that made by the burning method mentioned above (column *B*). The exposure for both sets of records was made on the same roof. In column *C* are the official figures of the Dominion Meteorological Station at Winnipeg. This station is about ten miles due north of the Manitoba Agricultural College, where this investigation was carried on. Since the clouds at this time of the year are usually of a general nature, it was thought that these records might be useful as a check.

TABLE I

COMPARATIVE RECORDS OF THE HOURS OF BRIGHT SUNSHINE AT MANITOBA AGRICULTURAL COLLEGE

Date 1931	*A* Photo-electric cell and electric clock		*B* E. R. Watts & Co. Sunshine Recorder	*C* Dominion Station records at Winnipeg
	hr.	min.	hr.	hr.
Jan. 1	0		0	0
Jan. 2	0	35.5	0	0.3
Jan. 3	7	25.0	6	6.2
Jan. 4	0		0	0
Jan. 5	7	35.5	6	6.2
Jan. 6	2	24.0	1.2	1.0
Jan. 7	0		0	0
Total	18	0.0	13.2	13.7

An attempt was made by the writer to make personal observations of the time of appearance and disappearance of the sun during these days. These observations are produced herewith.

January 1. No appearance of the sun.

January 2. The sun shone with full brightness from 4 p.m. until 4.35 p.m.

January 3. The sun appeared above a cloud bank in a clear sky at 8.45 a.m. and disappeared behind a dense, sharply defined cloud bank at 4.12 p.m. The sunshine recorder started burning at 9.40 a.m. and stopped at 3.35 p.m.

January 4. No appearance.

January 5. The sun became visible at 9.00 a.m. in a clear sky and disappeared behind a dense, sharply defined cloud bank at 4.35 p.m. The sunshine recorder started burning at 10.15 a.m. and stopped at 4.15 p.m. The glass sphere on the recorder was covered with a heavy coating of frost, which prevented its operation as early in the morning as it might otherwise have done. It might well be noted here that the recorder of the Dominion Meteorological Station at Winnipeg, started burning at about 10.30 a.m. its lateness in starting being due, without doubt, to a similar coating of frost.

January 6. The sun became visible at 9.15 a.m. and disappeared behind a cloud bank at 9.40 a.m. Between 9.40 a.m. and 4.40 p.m. the sun came through clear of all clouds on twelve different occasions, and shone brightly for periods of time varying in length from 3 to 45 min. The clock registered 2 hr. 23 min. A reproduction of the card from the sunshine recorder on this day appears in Fig. 1, C., and shows eight of these successive periods of sunshine for an estimated total of 1.2 hr.

On days under observation during December and January, when the sun has shone continually in a clear sky, a registration by the usual method has not included a record of the first and last half hour of sunshine, and in several cases the omission has totalled nearly two hours. The photo-electric cell and clock method has on no occasion thus far omitted any portion of the daily record that would not be considered questionable and on several days the clock has continued to operate until only half the sun was visible above the horizon.

Discussion

No attempt has been made in this proposed method, to record the actual time of day that the sun was visible. Such a record is of minor importance compared to that of the total daily hours. The call for such information is usual only when, for some reason, it is desired to recall the weather conditions on a certain day in the past.

The usual method of recording the hours of sunshine consists of measuring, somewhat inaccurately only one of its effects; namely, heat, as indicated by its ability when concentrated to char a suitably prepared card. This method is founded on the assumption that sunlight and heat are synonymous. Without entering into a discussion concerning the extent to which this is warranted for strictly practical purposes, it is submitted that sunlight is capable of measurement as luminous flux, more quickly and more accurately than the usual method indicates.

Of foremost importance in the success of this method of recording hours of sunshine is the selection of a minimum intensity acceptable as bright sunshine. Three alternatives are offered—the degree of illumination at sunset in a clear sky on December 21, on September 21 or on June 21.

If the first of these alternatives is accepted, as was the case in this investigation, the number of hours recorded will show a large increase over the usual method. It is also quite probable that a value above this minimum may be attained in the presence of some cloudiness during the summer months.

If the relay were adjusted to stop the clock at sunset on a day near the end of June, then it is probable that by late fall the loss in the record each morning and evening would be in a matter of a very few minutes. Observations show, as would be expected, that the indirect light finding its way into the cell exposed in this manner produces a very small effect compared to the direct rays of the sun. The tendency will be then for the clock to continue to run as long as the sun is visible.

The conclusion would be that it becomes important to have a definition of the minimum intensity of "bright sunshine".

Alternatively, if the record is intended to indicate the length of time the sun was actually visible, it would occasionally be necessary to alter slightly the adjustment of the relay as the length of day changed.

The investigation described in this paper is being continued.

Acknowledgment

The writer desires to acknowledge his indebtedness to Dr. Alfred Savage, Department of Bacteriology and Animal Pathology, Manitoba Agricultural College, for his interest and helpful suggestions.